BCL2 EXPRESSION IN DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH OTHER CLINICOPATHOLOGIC VARIABLES

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

In

PATHOLOGY – BRANCH III

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

MAY 2018

DECLARATION

I hereby declare that the dissertation entitled **"BCL2 EXPRESSION IN DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH OTHER CLINICOPATHOLOGIC VARIABLES"** is a bonafide research work done by me in the Department of Pathology, Coimbatore Medical College during the period from JULY 2016 TO JUNE 2017 under the guidance and supervision of Dr.V.PRABHA, M.D, Associate Professor, Department of Pathology, Coimbatore Medical College.

This dissertation is submitted to The Tamilnadu Dr.MGR Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree(Branch III) in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any Degree.

Place: Coimbatore **Dr. G.SHARMILA**

Date :

CERTIFICATE

This is to certify that dissertation entitled **"BCL2 EXPRESSION IN DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH OTHER CLINICOPATHOLOGIC VARIABLES"** is a bonafide work done by **Dr. G. SHARMILA**, a postgraduate student in the Department of Pathology, Coimbatore Medical College, Coimbatore under guidance and supervision of **Dr. V.PRABHA, M.D**, Associate Professor, Department of Pathology, Coimbatore Medical College, Coimbatore in partial fulfillment of the regulations of the Tamilnadu Dr. M. G. R. Medical University, Chennai towards the award of M. D. Degree (Branch III) in Pathology.

Guide

Dr. V.PRABHA, M.D,

Associate professor,

Department of Pathology,

Coimbatore Medical College

Coimbatore.

Head of the Department

Dr.C.Lalitha,M.D,

Professor,

Department of Pathology,

Coimbatore Medical College

Coimbatore.

Prof. Dr.B.ASOKAN M.S.,MCh.,

Dean

Coimbatore Medical College, Coimbatore

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : DR. G. SHARMILA

Course : M.D. PATHOLOGY

Period of Study : ONE YEAR

College : COIMBATORE MEDICAL COLLEGE

Dissertation Topic : Bcl 2 EXPRESSION IN BREAST CARCINOMA AND ITS ASSOCIATION WITH CLINICOPATHOLOGIC VARIABLES

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not~~ ac~~cepte~~d and you are permitted / ~~Not~~ p~~ermitte~~d to proceed with the above Study.

Member Secretary
Ethics Committee

16.3.16.

ACKNOWLEDGEMENTS

To begin with, I thank the almighty God for bestowing his blessings on me and making this dissertation a successful one.

I wish to thank our Dean **Dr.B.ASOKAN**, **M.S., MCH.,** Coimbatore Medical College and hospital, Coimbatore for permitting me to conduct this study.

I wish to thank our beloved Professor and Head of the Department, **Dr.C.LALITHA** MD, Department of Pathology, Coimbatore Medical College for her guidance and support.

I wish to express my gratitude and sincere thanks to **Dr. A. ARJUNAN**. M. D., for his guidance and support.

I express my gratitude and sincere thanks to my guide **Dr. V. PRABHA**, M.D, Associate Professor, Department of Pathology, Coimbatore Medical College, Coimbatore. This dissertation bears her valuable suggestions and highly professional advice.

I owe my gratitude **Dr. A. DHANALAKSHMI** M. D., and **Dr.G.S.THIRIVENI BALAJJI**, M.D., for their timely advice and suggestion through the course of my work.

I would like to thank all my teachers for their guidance and valuable advice not only regarding my thesis work but also throughout my graduate experience.

I thank Department of Surgery and Department of Surgical Oncology Coimbatore Medical College, Coimbatore for providing clinical cases, valuable support and guidance which made this dissertation possible. I thank all lab technicians working in Department of Pathology and all my patients for their co-operation and support.

At this juncture, I have a special mention about my family members without them I couldn't travel this far. So I would like to dedicate this book to my parents- Mr.R.Gunasekaran & S.Santha, my husband Mr.S.Karthick and my lovable princess CHAARU NETIRAA & prince charm GOWTHAM ARJUN KRISHNAA. I would also like to thank my sister Mrs.Vennila and my mother in law Mrs.Tamilarasi for their esteemed support and prayers.

I also thank all my friends, my fellow batchmates, my juniors for their love and support and atlast above all, I would like to dedicate this work to my dear **GAYU** and my grandmother. It would be nice if they are with me now…..

Dr.G.SHARMILA

URKUND REPORT

URKUND

Urkund Analysis Result

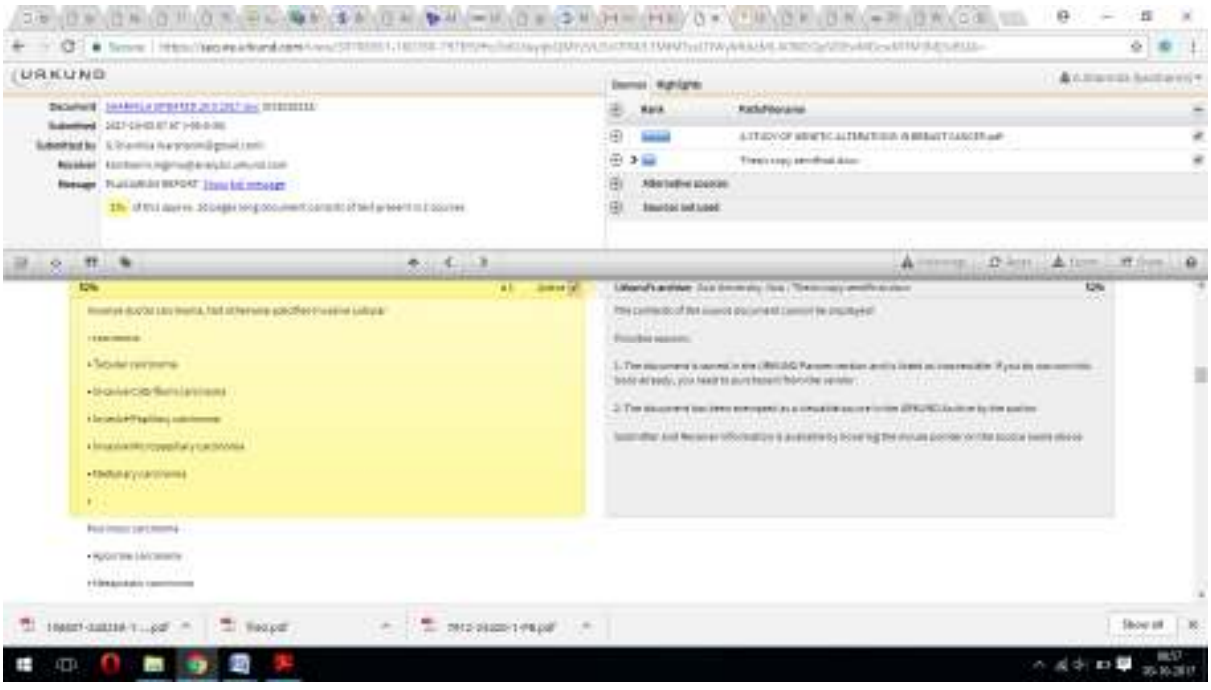
Analysed Document: SHARMILA UPDATED 29.0.2017.doc (D31028232)
Submitted: 10/5/2017 3:37:00 AM
Submitted By: karsharmi@gmail.com
Significance: 1 %

Sources included in the report:

Thesis copy semifinal.docx (D30605160)
A STUDY OF GENETIC ALTERATIONS IN BREAST CANCER.pdf (D22605196)

Instances where selected sources appear:

2



CERTIFICATE – II

This is to certify that dissertation work titled **BCL2 EXPRESSION IN DUCTAL CARCINOMA OF BREAST AND ITS ASSOCIATION WITH OTHER CLINICOPATHOLOGIC VARIABLES** of the candidate **Dr.G SHARMILA** with registration number **201513255** for the award of **M.D Degree** in the branch of **PATHOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** percentage of plagiarism in the dissertation

Guide and Supervisor sign with seal

INDEX

LIST OF TABLES

LIST OF CHARTS

LIST OF COLOUR PLATES

S.No	TITLE
1.	Immunohistochemical expression of bcl2 in mucinous carcinoma of breast
2.	Immunohistochemical expression of bcl2 in ductal carcinoma of breast
3.	Immunohistochemical expression of ER positivity
4.	Immunohistochemical expression of PR positivity
5.	Immunohistochemical expression of Her2 overexpression
6.	Immunohistochemical expression of bcl2 negativity
7.	H&E picture of invasive ductal carcinoma of breast
8.	H&E picture of mucinous carcinoma of breast
9.	H&E picture of secretory carcinoma of breast
10.	H&E picture of metaplastic carcinoma of breast

INTRODUCTION

Breast cancer has now become the most common cancer among Indian females exceeding cervical cancer. Age adjusted incidence rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. Trends of breast cancer now in India are increasing incidence at younger age group, late stage of presentation, aggressive cancers in the young. The major factors underlying this trend in developing countries are thought to be social changes that increase breast cancer risk, specifically - delayed child bearing, fewer pregnancies and reduced breast feeding, combined with a lack of access to optimal health care.

Almost all breast malignancies are adenocarcinomas (>95%). In the most clinically useful classification system, breast cancers are divided based on the expression of hormone receptors-estrogen receptor(ER) and progesterone receptor(PR) and the expression of human epidermal growth factor receptor2(HER2,also known asERBB2),into three major groups:

- ER positive(HER2 negative;50-65% of cancers)
- HER2 positive(ER positive or negative;10-20% of cancers)
- Triple negative(ER,PR,HER2 negative;10-20% of cancers)

These three groups show striking differences in patient characteristics, pathologic features, treatment response, metastatic patterns, time to relapse and outcome. Within each group are histologic subtypes, some of which

also have clinical importance. The prognosis and treatment of breast carcinoma depends on tumour size, histological grade, lymphnode stage, expression of estrogen receptor, progesterone receptor, over expression of her2/neu. Gene expression profiling(GENE SIGNATURE) is becoming standard care for breast cancer patients, but is available in selected institutions and is subject to issues like feasibility, interpretation and cost. These are of critical relevance in considering the need to identify molecular features of individual tumours in routine practice.

This includes ER,PR,HER2,P53,KI67.In addition markers of angiogenesis and apoptosis are used. Identifying prognostic molecular markers recently has become the objective in translational research studies on breast carcinoma. These markers potentially could serve as a complement to clinico pathologic staging in identifying patients who have high risk for disease recurrence and who need systemic therapy. Bcl 2-a protooncogene identified in 1984. The protein is found mainly in the periphery of mitochondria, on the perinuclear membrane, and in the endoplasmic reticulum.

About 70% of cases of follicular lymphoma and 20% of cases of diffuse B-cell lymphomas have increased concentrations of Bcl-2 protein as a result of the t(14;18)(q32;q21) translocation which positions the Bcl-2 gene under the control of the strong Ig heavy-chain promoter gene. The fused Bcl-2-Ig gene generates chimeric mRNAs that consist of Bcl-2 at 5' portion

and immunoglobulin at 3' portion, and the chimeric mRNA contains the Bcl-2 coding frame for a 239-amino acid polypeptide. In consequence, high levels of Bcl-2 protein are generated. However, a discrepancy in the relationship between the occurrence of t(14;18) translocation, Bcl-2 gene rearrangement, and over expression of Bcl-2 protein has been found in lymphoma, which suggests the existence of several molecular mechanisms for Bcl-2 protein over expression. A number of investigators have shown that bcl2 is a candidate prognostic marker. In our study we read about bcl2 expression in different histologic types of breast carcinoma and correlates its expression with other clinico pathologic variables such as tumour size, histological grade, lymph node status, hormone receptor status and Nottingham prognostic index.

AIM OF THE STUDY

To assess Bcl2 expression in breast carcinoma and its correlation with other clinicopathologic factors such as tumour size,lymph node status,histological grade,ER, PR and HER2/ neu expression and Nottingham prognostic index.

OBJECTIVES

- To analyze Bcl2 expression in invasive ductal carcinoma of breast

- To correlate Bcl2 expression with ER, PR and HER2 neu expression.

- To analyze the correlation of histological grade, Nottingham prognostic index of each case with Bcl2 expression.

REVIEW OF LITERATURE

ANATOMY

The breast (mammary gland) is a subcutaneous glandular organ of the superficial pectoral region. It is a modified sweat gland, especially in women for production and secretion of milk. A variable amount fat surrounds the breast tissue which is responsible for the shape, size of the breast.

Nipple

The nipple contains the opening of the lactiferous ducts. It is located at the level of fourth intercostal space in nulliparous women and men. It contains circular smooth muscle fibers which contract during ejection of milk from ducts.

The areola is a area surrounding the nipple, it contains sebaceous glands. There are 15 to 20 lactiferous ducts, each drains a lobule of breast. These ducts radiates outward from the nipple. The terminal portion of each duct called the lactiferous sinus, is dilated.

Cooper's Ligaments

These are suspensory ligaments , which attach the mammary gland to skin and from skin to deep fascia.

Arterial Supply

Most of blood supply to the breast is from branches of the internal thoracic artery. Lateral thoracic and thoracoacromial branches of axillary artery and intercostal arteries also contribute to it.

Venous Drainage

Venous blood from breast drains primarily through the axillary vein

.

Lymphatic Drainage

Most of the breast drains to axillary nodes . Lymphatics from the deep surface drain to apical group of axillary nodes, the medial surface drains to the parasternal nodes.

BREAST DEVELOPMENT

The female breast undergoes dramatic changes in size, shape and function during growth , puberty, pregnancy, lactation, and postmenopausal regression. Lobular formation occurs at puberty but completion of development and differentiation occurs only at end of a full-term pregnancy. With pregnancy, the mammary parenchyma reaches its final stage of development with secretory lobules. It is postulated that induction of differentiation of breast by pregnancy is

partially responsible for inhibition of carcinogenic initiation. Breast is composed of 15–20 segmented units. Large ducts progressively branch into smaller ducts that end as terminal ductal lobular units (TDLU) Histologically, mammary epithelium is ectodermally derived and is composed of inner epithelial cell layer and outer myeoepithelial layer . The epithelial cells are hormonally responsive .

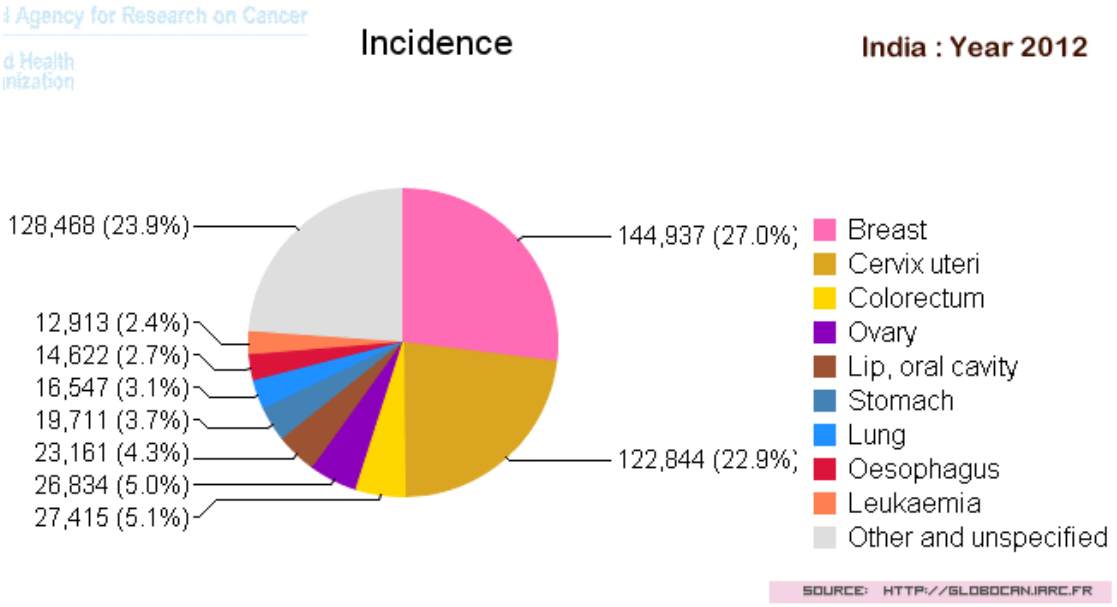
Microscopic Anatomy of the Adult Breast

The superficial portion of duct orifice is lined by squamous cells. The squamocolumnar junction, where squamous epithelium joins glandular duct epithelium, is normally referred as the lactiferous sinus. Extension of squamous epithelium into or beyond the lactiferous sinus is termed as squamous metaplasia . This results in obstruction of the affected duct The squamocolumnar junction is an important landmark in pathogenesis of Paget's disease.

Cells of ductal epithelium are of two types. They are columnar / cuboidal cells lining the lumen. Myoepithelial cells lie between the epithelium and basal lamina. Spindle-shaped myoepithelial cells lie parallel to long axis of the duct and form a continuous layer. The histologic appearance and immunoreactivity of myoepithelial cells are variable, especially in pathologic conditions.

INCIDENCE AND EPIDEMIOLOGY

Carcinoma affects all communities worldwide. Breast cancer is most common non skin malignancy in women . In India according to National cancer registry programme 2014 report,there is increased incidence of breast cancer as high as 25.8 per 100,000 women.Mortality rate for breast cancer in India is also high-12.7 per100,000 women.[1]

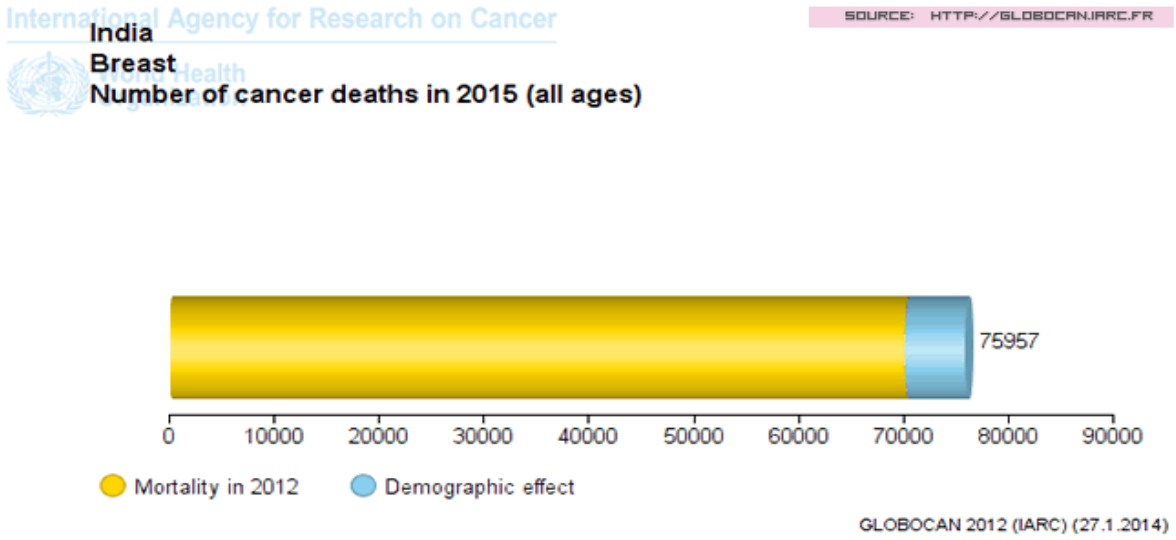


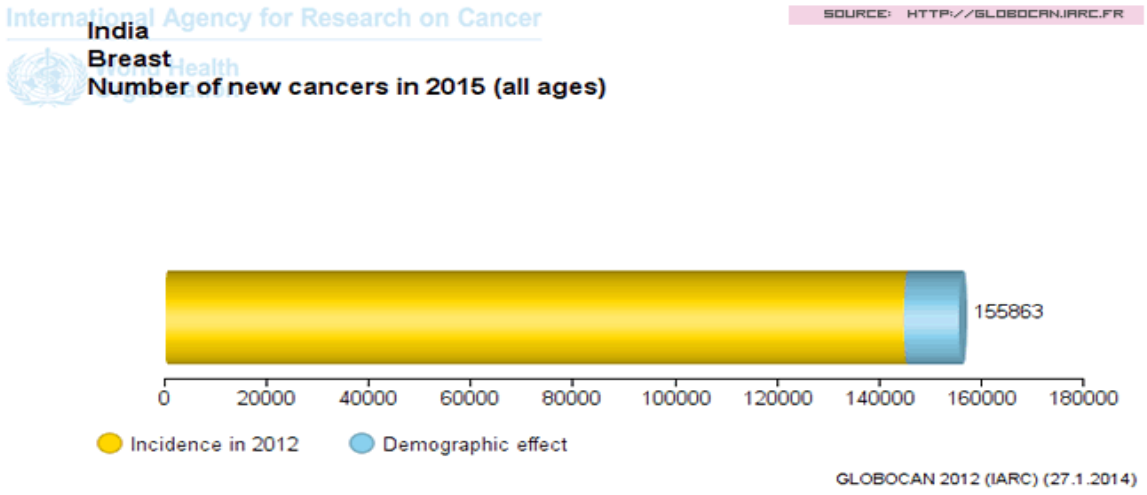
WHO prediction for breast cancer in India

The upper image below shows the prediction of numbers of deaths due to breast cancer in 2015 and the lower image shows the predictions of the numbers of newly detected cases of breast cancer.

For the years 2018, there will be an estimated 1,55,000 new cases of breast cancer and about 76000 women in India are expected to die of the

disease. The gap only seems to be widening, which means, we need to work aggressively on early detection.[1]





Breast cancer appears to be increased due to screening programme. The major benefit of mammographic screening is detection of insitu carcinoma and to avoid large number of cases presenting in advanced stage. In India incidence of the breast cancer is more common among fourth to fifth decades of age .Breast carcinoma mortality is brought down due to the diagnosis at early

stage itself and because of effective screening and advanced therapeutic methods.[2]

Despite the fact that with early detection, actually more woman are surviving breast cancer. The use of adjuvant therapy in breast carcinoma is controversial because the response rate in patients with metastatic breast carcinoma on combination chemotherapy regimen are 50-60% with complete remission only in 10-15% of individuals . A major reason for failure in treatment is inherent/acquired drug resistance of tumour.Therefore understanding the pathophysiology of disease is critical for identifying novel therapeutic stratagies to improve patient outcome.

Apoptosis(programmed cell death type1) and Autophagy (type2) are crucial mechanisms involved in regulating cell death, maintaining internal homeostasis and thereby eliminating unwanted/malignant cells. Elimination of cancer cells following chemotherapy occurs in part through the induction of apoptosis.Recent finding suggest that Bcl2 blocks not only apoptosis also autophagic cell death in breast carcinoma.[3]

Bcl 2, first discovered in Bcell malignancies ,is located in chr 18q21.33. Family of Bcl2 proteins are mitochondrial proteins which regulate apoptosis .By forming complex homodimers and heterodimers these proteins either inhibit or accelerate apoptosis.[4] Bcl2 blocks apoptosis through mitochondrial pathway by inhibiting the release of cytC from mitochondria which leads to caspase9 activation

and subsequent apoptosis**. Bcl2 shown to inhibit chemotherapy induced apoptosis and chemotherapy resistance has been reversed in cancer cells treated with targeting therapy**. Although Bcl2 is an antiapoptotic protein, high Bcl2 expression has been observed in ER positive cancers.

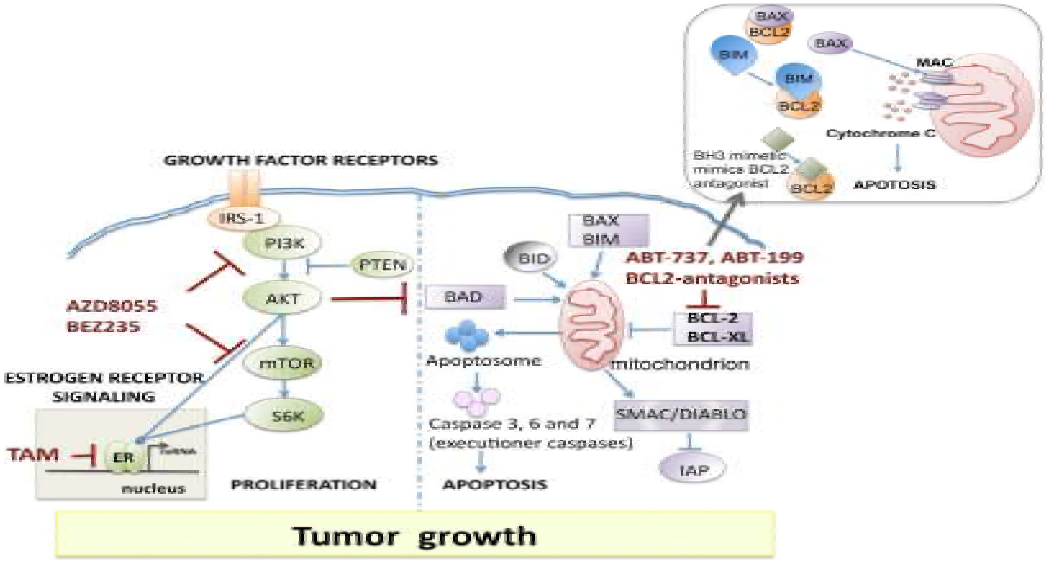
Interplay between ER Signaling and Apoptosis

Estrogen receptor (ER) is regulated by estrogen binding and phosphorylation by a number of signal transduction kinases, including AKT. The activated ER dimerizes and binds classically to estrogen response elements on target genes controlling proliferation and survival. The gene which encodes the antiapoptotic protein BCL-2 is an estrogen-regulated gene. Tamoxifen, competes with estrogen for the estrogen receptor (ER) and suppresses its transcription by inducing the recruitment of corepressors as opposing to coactivators, leading to reduction in cell proliferation.

Apoptosis is driven by a complex interplay between the proapoptotic and antiapoptotic proteins.[5,6,7] BAX/BAK induce apoptosis by forming pores in the outer membrane of the mitochondria (mitochondrial apoptosis-induced channel [MAC]), leading to the release of cytochrome c, which activates the cytoplasmic caspase cascade. BCL-2 ,BCL-Wand BCL-XL are antiapoptotic proteins that block BAX/BAK activity.[8]

The antiapoptotic function of BCL-2,BCL-W and BCL-XL are held in check by the apoptotic proteins BIM/BAD. Both BIM and BAD can form heterodimers with BCL-2, thus releasing BAX. BAD is a phosphorylation target of AKT. When phosphorylated, it is inactivated, releasing BCL-2 to inhibit BAX. This interplay between BCL2 and BIM/BAD has been exploited by development of the new BH3 mimetics, which modulates the interaction between BCL-2 and BIM/BAD, leading to the release of BAX and induction of apoptosis

Interplay between ER Signaling and Apoptosis



In preclinical studies ,bcl2 protein inhibits apoptosis in vitro and is associated with chemoresistance. But in vivo ,the expression of bcl2 protein should inhibit apoptosis thereby implies worse outcome. To surprise ,statistics in previous studies revealed that bcl2 positive patient had better prognosis and better survival compared with bcl2 negative tumours. Prognostication of breast cancer using clinicopathologic variables

although useful remains imperfect.Gene expression profiling or gene signature assays can refine the current approach.[9,10]Alternatively panel of proteins assessed by IHC may be useful.Hence in our study we evaluate the expression of bcl2 in various histologic types of breast carcinoma, in the absence of advanced molecular biological techniques by means of immunohistochemistry.

MARKERS OF PROGNOSIS AND RESPONSE TO THERAPY

This panel of markers includes ER,PR,HER2,P53,Ki67.In addition markers of angiogenesis(VEGF,Angiogenin,Angiopoetin-1,TGF) and apoptosis(Bcl2,Bcl-XL,Bcl-W,MCL-1) are used. In breast carcinoma patients,bcl2 expression is associated with hormone receptor status and usually associated with a non aggressive low grade slowly proliferative ER+ breast cancer. Since bcl2 is over expressed it suggest that it contributes to tumour initiation, progression, and resistance to therapy. Hence bcl2 targeting therapy may be effective for treatment. We also studied the role of bcl2 as a surrogate predictive marker of response to neo adjuvant chemotherapy.[11]

Naoko honma et al.BMC cancer 2015 stated that Bcl2 expression is an independent poor prognostic factor in patients with hormone receptor negative or triple negative breast cancers, especially in the absence of adjuvant therapy.Bcl2 antisense therapy has been suggested for various tumours in an in vitro setting. The development of bcl2 inhibitors

has been explored and small molecule inhibitors such as ABT-737 AND ABT-199 have been recently introduced. Emerging evidence also suggests the usefulness of this type of therapy in breast cancer.[12]

Dana Carmen Zaha et al in ROM J MORPHOLEMBRYOL 2012 stated that Bcl2 positive tumours represent 92.3% of all luminal A tumours and 60% of luminal B .Instead non luminal tumours(basal like, unclassified and HER2) are negative. Five year survival rates were significantly better for Bcl2 positive patients and worse in patients with Bcl2 negative expression.[13] Mee Seon Kim et al in Int J Clin Exp Pathol 2015 stated that breast cancer is a heterogeneous disease,it is important to select patients who should undergo adjuvant therapy and predict their prognosis.They found out in their study that overexpression of BCL2 in breast cancer has been associated with favourable prognosis.[14]

Abdel fatah et al in Annals of oncology june 2013 studied about Bcl2 expression in triple negative breast cancer(TNBC).[15]TNBC traditionally considered to have a poor prognosis.Neither the classical pathological variables nor the modern molecular assays have shown the prognostic value in this group. Current research focused on the identification of biological markers that could be used to tailor the treatment to individual patients with TNBC. Their study show Bcl2 to be a promising prognostic and predictive marker in TNBC. They also shown that Bcl2 status could help predict the outcome of treatment.

Ali and Coombes et al in a study 2002 says that Estrogen-bound ER associates classically with Estrogen response elements on target genes controlling proliferation and cell survival.[16] Teixiera et al(1995) reported that MCF7 human ER+ breast cancer cells had increased sensitivity to the cytotoxic agent doxorubicin when treated with antisense BCL-2.[17] Now recently, BH3 mimetic small molecules, which mimic the action of proapoptotic BH3-only proteins, has been developed which counteract antiapoptotic proteins such as BCL-2 and BCL-2-related proteins BCL-XL and BCL-W

Samantha R Oakes et al in a study 2012 says that Basal like tumours account for 20% of breast cancer and express basal markers such as cytokeratin5/6 and epidermal growth factor receptor. They report increased expression of Bcl2 in various subtypes including basal type and revealed that ABT-737 potentiates the effects of docetaxel chemotheraphy in basal like breast cancers with elevated levels of Bcl2,suggesting BH3mimetics in combination chemotherapy as a potential treatment for this aggressive cancer subtype.[18] Ki Tae Hwang et al published a study in International journal of cancer 2012 about Prognostic influence of Bcl2 expression in breast cancer[19]. In their study they found Bcl2 was a powerful independent prognostic factor for breast cancer. Favourable clinico pathological features and strong correlation with hormonal receptor

are suggested as causes of superior survival in patients with Bcl2 positive breast cancer.

Fisnik Kurshumliu et al. World Journal of Surgical Oncology 2014,demonstrates that expression of ER,PR,and Bcl2 is seen with higher frequency in good and moderate NPI GROUPS.[20]In contrast, over expression of HER2/neu is more frequent in moderate and poor NPI groups.NPI(Nottingham prognostic index) is a numerical value that is calculated by adding the values of tumour diameter(multiplied by coefficient of 0.2),histological grade (1 to 3),and lymph node stage(1 to 3).It's a three tiered classification system distinguishing good, moderate, and poor prognostic groups with cut-off points between the values <3.4,3.4 to 5.4,>5.4.

IN OUR STUDY WE READ ABOUT BCL2 EXPRESSION IN BREAST CANCER AND ATTEMPT TO VALIDATE THE ROLE OF BCL2 AS A PROGNOSTIC FACTOR OF BREAST CANCER.

Risk factors for breast carcinoma

Gender is one of the important risk factor, because only one percent of breast carcinoma occurs in men.

Age

Breast cancer may occur at any age . Most of all cases occur in reproductive age group . It is rare below twenty five

years . More than 60% of breast cancer occurring in young people are either ER/PR negative or else HER2/neu positive.[21] Early age at menarche and late menopause are the risk factors for breast cancer. Females whose first term pregnancy was at early age , below twenty has less risk than those women over thirty five years of age or nulliparous women .

It is because the pregnancy causes terminal differentiation of breast luminal cells and thereby remove the potential for precancerous cells. Positive Family history of breast cancer among the first degree relatives increases the risk of occurrence in that individual. The risk occurs due to germline mutation in BRCA1 and BRAC2 . These genes are responsible for only 16% of familial cancer. Affected individuals has to be closely followed up or they can do prophylactic mastectomy.

Epithelial hyperplasia and fibrocystic disease

Presence of Atypical ductal hyperplasia , fibrocystic disease increases the risk of carcinoma breast.

Estrogen exposure

There is 1.7 fold of increased risk with associated hormonal replacement therapy (estrogen and progesterone). Those cases are mostly ER positive and of invasive lobular carcinoma.

Density

XRAY showing Increased radiodensity is also a risk factor for breast cancer. It correlates with young age and hormonal exposure. Routine screening programme is not effective here. In some cases MRI may be of use.

Radiation

Radiation exposure of any form are associated to the increased incidence of breast cancer

Geography

Incidence in USA and Europe are 7 times high when compared to other countries. But nowadays the incidence of number of cases in India is alarming.

Dietary risk

Consumption of caffeine may lower the risk but alcohol intake may increase the risk of breast cancer.

Obesity

In obese individual the risk is low when the age is below 40 due to lower progesterone level. In postmenopausal obese women the risk is increased due to excess estrogen synthesis in fat.

Exercise

Risk factor for breast carcinoma are slightly lower for the physically active women than the inactive one.

Breast feeding

Increased reduction of risk for breast cancer noted among women who breastfeed their child for longer periods. Lower the incidence of breast cancer in developing countries may be due to frequent and longer breast feeding.

ETIOLOGY AND PATHOGENESIS

Breast carcinomas are clonal proliferation of cells with multiple genetic abnormalities which are further influenced by genetics and hormonal exposure. Around 10 % of all breast cancer is due to a inheritance of germline mutations in tumour suppressor gene. Multiple affected first degree relative, breast cancer at younger age group, incidence of multiple cancer in the family - suggests hereditary etiology. The major susceptibility genes for hereditary breast cancer is BRCA1,2,TP53 AND CHEK2 which has role in repair of DNA and thereby maintaining genomic integrity. Mutation of highly penetrant gene is the cause for increased risk.

BRAC1 and BRCA2 contributes to 80-90% of Inherited forms of breast cancer.[22] BRCA1 located on chromosome 17q21 is associated

with risk for ovarian carcinoma. BRCA2 gene on chromosome 13q12.3 is associated with male breast cancer. Mutation of both the genes are associated with pancreatic and prostatic cancer. Both BRCA1 and BRCA2 are larger genes and has different spectrum of mutation in their coding region. Functions include recombination DNA repair, cell cycle control, chromatin remodeling. Protein encoded by BRCA2 gene also involved in DNA repair and cytokinesis.

BRCA1 mutated breast cancer are usually poorly differentiated tumour having medullary carcinoma features and triple negative phenotype or overexpression of HER2/neu,basal like type of tumour. BRCA2 mutated tumours are often ER+, poorly differentiated tumours. There are other susceptibility genes which are rarely associated with familial breast cancer. It includes

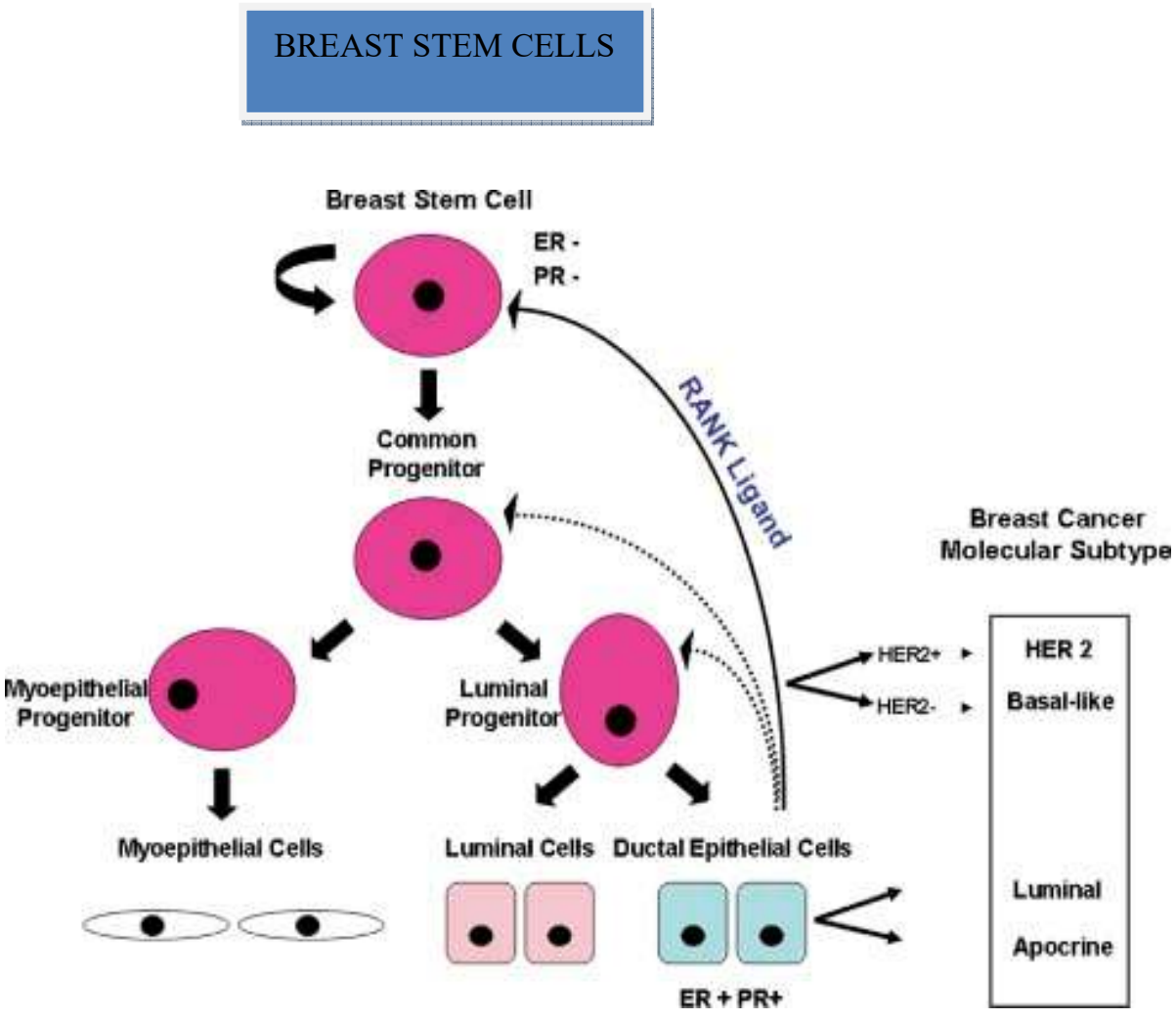
1. Germline mutation in p53
2. Mutation in CHEK2 which totally accounts for about 8% of breast carcinoma. Other susceptible tumour suppressor genes are
3. PTEN Gene-Cowden syndrome
4. STK11-Peutz jeghers syndrome
5. ATM Gene-ataxia telengiectasia.

ATM senses damage of DNA with help of p53 and CHEK2 causing cell cycle arrest.BRCA1, BRCA2, CHEK2 all together helps in repair of DNA through homologous recombination.[23,24]

Sporadic Breast Cancer

Majority of sporadic cancer is due to their hormonal exposure- gender, age at menarche and at menopause, reproductive history of individual, breast feeding and history of any hormone therapy . Most of them are postmenopausal females and are ER positive. Hormonal exposure indeed causes increased proliferation of target cells, proliferation increase the chances of DNA damage thereby increased incidence of carcinoma.[25,26]

MOLECULAR MECHANISMS OF CARCINOGENESIS AND TUMOUR PROGRESSION



Classification

- More than 95% of breast cancers are adenocarcinomas. They are divided into in situ carcinomas and invasive carcinomas.[29] Neoplastic proliferation that is restricted to ducts and lobules is called as in situ carcinomas. When penetration occurs to stroma through basement membrane it is called as invasive carcinoma.

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE BREAST[28]

A) EPITHELIAL TUMORS:

- Invasive ductal carcinoma, Not otherwise specified Invasive Lobular
- carcinoma
- Tubular carcinoma
- Invasive Cribriform carcinoma
- Invasive Papillary carcinoma
- Invasive Micropapillary carcinoma
- Medullary carcinoma
- Mucinous carcinoma
- Apocrine carcinoma
- Metaplastic carcinoma
- Secretory carcinoma
- Neuro Endocrine carcinoma

- Oncocytic carcinoma
- Adenoid cystic carcinoma
- Acinic cell carcinoma
- Glycogen rich clear cell carcinoma
- Sebaceous carcinoma
- Inflammatory carcinoma
- Micro invasive carcinoma

INTRADUCTAL PROLIFERATIVE LESIONS

- Usual ductal hyperplasia
- Flat ductal hyperplasia
- Atypical ductal hyperplasia

- Ductal carcinoma in situ
- Intraductal papillary neoplasm
- Central papilloma
- peripheral papilloma
- Intraductal papillary carcinoma
- Benign epithelial proliferation

Adenosis and its variant

Radial scar or complex sclerosing lesion

Adenomas and its variants.

B) MYOEPITHELIAL LESIONS:

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

C) MESENCHYMAL TUMORS:

- Hemangioma
- Angiomatosis
- Hemangiopericytoma
- Myofibroblastoma
- Fibromatosis
- Inflammatory myofibroblastoma
- Lipoma
- Leiomyoma
- Granular cell tumor
- Neurofibroma
- Schwannoma
- Angiosarcoma
- Liposarcoma
- Rhabdomyosarcoma
- Osteosarcoma

- Leiomyosarcoma

D) FIBROEPITHELIAL TUMORS:

- Fibroadenoma
- Phyllodes tumor
- Periductal stromal sarcoma
- Mammary Hamartoma

E) TUMORS OF NIPPLE:

- Nipple adenoma
- Paget's disease of nipple.

F) MALIGNANT LYMPHOMA:

- Diffuse Large B cell Lymphoma
- Burkitt Lymphoma
- Extra nodal marginal zone B cell Lymphoma of MALT type
- Follicular Lymphoma.

G) METASTATIC TUMORS

H) TUMORS OF MALE BREAST:

- Gynaecomastia,
- Carcinoma- invasive and in situ

DUCTAL CARCINOMA IN SITU (DCIS)

Detection of DCIS is increased due to mammography which detects calcification and less commonly periductal fibrosis. It may spread throughout the duct. Morphological variants of DCIS

- Comedocarcinoma
- Solid
- Cribriform
- Papillary
- Micropapillary
- Clinging
- Cystic hypersecretory.

Macroscopy

DCIS may have an ill defined area of fibrous tissue. Soft, pale cheese like necrotic debris can be seen on cut surface in comedocarcinoma. Most of small lesion are invisible.[29]

Grading

Using nuclear grade DCIS is divided into three grades

- High
- Intermediate
- Low

High grade

This consists large pleomorphic cells and high nucleocytoplasmic ratio. Nucleus is enlarged, have coarse chromatin and large nucleoli. Frequent and atypical mitosis are seen. Comedo carcinoma, cribriform or micropapillary are the patterns associated with high grade DCIS.

Intermediate grade

Nuclei in this grade show less pleomorphism than high grade and they lack uniformity. Necrosis and nucleoli may be seen but they are not large. The architectural patterns includes cribriform, solid and micropapillary.

Low grade

This grade will have uniform cells with small regular nuclei. Nucleoli is indistinct. Necrosis and mitoses are uncommon. Cribriform, micropapillary and less commonly solid patterns are the patterns associated with low grade DCIS.

Rare variants

- Apocrine
- Neuroendocrine
- Signet ring cell
- Cystic hypersecretory

Untreated cases of DCIS turn into invasive cancer at a rate of 1% per year. Surgical excision followed by radiation is the usual treatment. Recurrence rarely occurs due to residual DCIS or occult foci of invasion.

LCIS

Lobular carcinoma in situ (LCIS), otherwise called as lobular neoplasia, is usually diagnosed as an incidental finding. It has no distinguishing features grossly. Bilateral LCIS is more common than DCIS. Lobular pattern is attributed to the loss of E cadherin, a transmembrane adhesion molecule, that causes cell to cell cohesion in normal breast.[30]

INVASIVE CARCINOMA

When stromal invasion is seen it is called as invasive carcinoma, whether in situ cancer present or not. If the invasion is less than 0.1cm it is called as **microinvasion**. Although the carcinoma of breast develops from stem cells, depending upon pattern of differentiation it is divided in to two major categories- ductal type and lobular type.

Clinical features

Most of the cancers present as a palpable mass, which are associated with axillary lymph node involvement in more than 50% cases. Fixation to the chest wall and skin dimpling can occur. Tumor involving the centre of breast causes nipple retraction. Blockage of lymphatics produces skin thickening and lymphedema. In such cases peau d' orange appearance occurs due to tethering of skin to the breast by cooper ligaments. With the introduction of mammographic screening small sized tumors are increasingly detected. They may present as radiodense mass and microcalcification .

Rarely axillary nodal metastasis or distant metastasis may be the mode of presentation of an obscured or occult primary breast cancer. At molecular level, breast cancer is classified based on DNA, RNA, and proteins of cancer breast.[31]

DISTRIBUTION OF HISTOLOGICAL TYPE

CARCINOMA IN SITU	**15-30%**
Ductal carcinoma in situ	80%
Lobular carcinoma in situ	20%
INVASIVE CARCINOMA	**70-85%**
No special type	79%
Lobular carcinoma	10%
Tubular carcinoma	6%
Mucinous carcinoma	2%
Medullary carcinoma	2%
Papillary carcinoma	1%
Metaplastic carcinoma	<1%

Invasive carcinoma no special type

70-80% breast cancers are invasive carcinoma of no special type. It fails to exhibit histologic characters to classify other specific types such as lobular, mucinous or tubular carcinoma.

Macroscopic Appearance

Most of them are firm to hard in consistency with irregular borders. The gross appearance varies. Size of tumor ranges from 0.5cm to above 10cm. On cutting the tumor there is gritty feeling due to presence of

small foci of elastic stroma and occasional calcification. High grade lesions are charecterised by presence of massive necrosis or calcification.

Histological appearance

The histological appearance of the tumour is variable. The tumor cells are arranged in sheets or cords. Sometimes they are diffusely infiltrative. Individual cells have abundant eosinophilic cytoplasm with pleomorphic nuclei. Glandular structure may be extensive or absent. Stromal component varies from desmoplastic proliferation to scanty connective tissue. Foci of elastosis and necrosis present which may be extensive. Features of special type of breast cancer is seen in variable proportion. Occasionally metaplasia, bizarre tumor giant cells are seen. Up to 80% cases are associated with DCIS component.[32]

Molecular subtypes

There are five major pattern of gene expression in no special type group. These are identified with Gene expression profiling. They have luminal A, luminal B, normal, basal like and HER2 positive.

Luminal A

It is the largest group and 40-55% of no special type cancers belonging to this group. Luminal A type of cancers are ER positive and HER2/neu negative. Most of the tumors are well or moderately

differentiated and mostly occur in post menopausal women. These tumors are slow growing and respond well to hormonal therapies.

Luminal B

15-20% of no special type cancer are luminal B type. It not only expresses ER but also often over express HER2/neu. These tumors are generally high grade. Most likely to have nodal spread. These tumors respond well to chemotherapy.

Normal breast like

6-10% cancers are normal breast like type. These are usually ER positive, HER2/neu negative well differentiated cancers. Their gene expression pattern is similar to normal breast.

Basal like

It accounts for 13-25% of no special type cases. These are characterized by ER, PR and HER2/neu negativity. They express markers of myoepithelial cells (P- cadherin, keratin, p63) or stem cells (cytokeratin 5 and 6).[33] Basal like cancers are subgroup of triple negative carcinomas. Most of the cancer occurring in women with BRCA1 mutation are basal like cancers. These are generally high grade and associated with poor prognosis. Only 15-20% respond to chemotherapy.

Her2 positive

It accounts for 7-12% of no special type cancers. This group over expresses Her2 protein. They are ER negative cancers. Amplification of a

segment of DNA in chromosome 17q21 causes over expression of Her2 neu.[47] These tumors are poorly differentiated and frequently have brain metastasis.

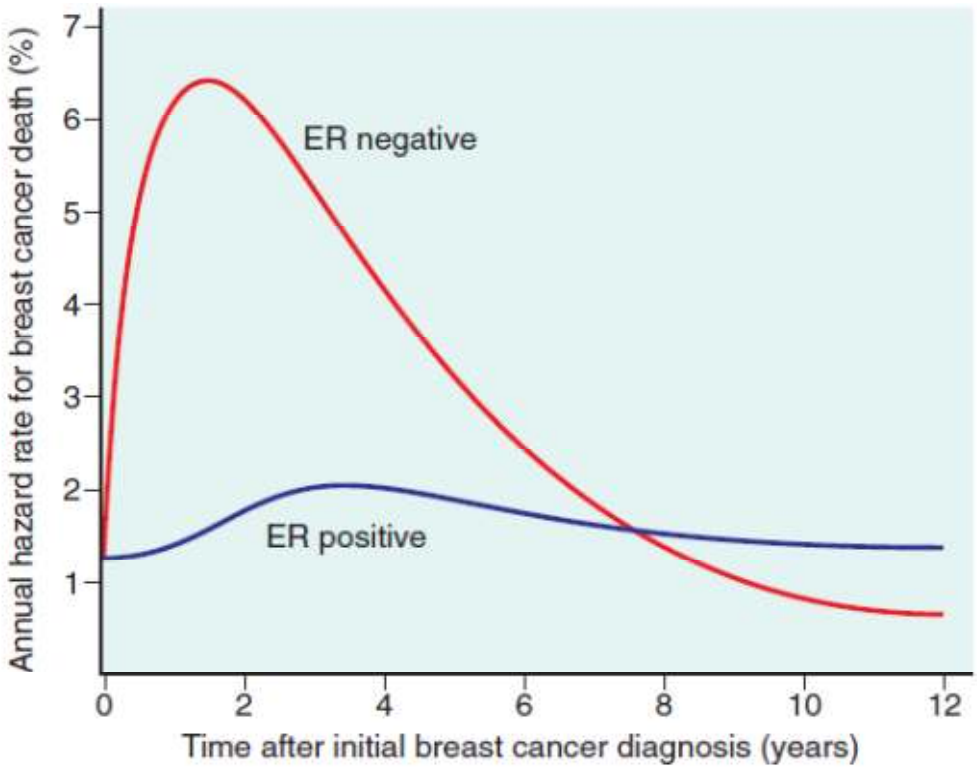


Fig. shows Time of recurrence of breast cancers. The hazard ratio reflects the risk of recurrence of each molecular type of breast cancer at various points in time after diagnosis. ER-negative cancers usually recur within the first 8 years. Patients who survive beyond this interval are likely cured. In contrast, ER-positive cancers have a lower rate of recurrence, but remain at risk decades after the primary diagnosis.

INVASIVE LOBULAR CARCINOMA

This occurs commonly in older age group than ductal type. They frequently present with larger tumor size, low histological grade and hormone receptor positivity. It is the most common special type of breast cancer. Higher rate of multicentricity, bilaterality, subsequent involvement of contralateral breast are frequently seen in invasive lobular carcinoma. Lobular morphology is due to loss of E-cadherin and loss of chromosome 16q.

Macroscopy

Most of the invasive lobular carcinoma forms a mass which is indistinguishable from ductal type. Rarely ill defined lesion may occur which is difficult to detect clinically and with imaging techniques.

Histopathology of classic type

This type accounts for 40% cases.[34] Small to moderately sized cells, arranged in dyscohesive cords, sheets, clusters or single file pattern are the hallmark features of this type. Concentric infiltration of tumor cells around normal ductular structures gives rise to targetoid appearance. Tumor cells are more or less uniform and have round to oval nuclei with inconspicuous nucleoli and a thin rim of cytoplasm. Nuclei often eccentrically placed. Intracytoplasmic lumina is commonly seen. Mitosis and desmoplasia are infrequent.

Other variants

- Alveolar
- Solid
- Tubulolobular
- Pleomorphic
- Mixed type.

Immunoprofile

They are commonly positive for ER, PR than in ductal type. HER2 positivity is lower than ductal type. They also express CEA. 10-16% cases express E-cadherin which is an uncommon finding. Metastatic pattern is different in lobular carcinoma and tend to involve the peritoneum, retroperioneum, leptomeninges, gastrointestinal tract and ovaries.

Tubular carcinoma

It is an uncommon subtype of breast cancer. Accounts for <2% of invasive breast cancer.[35] Other favourable features include smaller size, low lymph node metastasis and lower recurrence and excellent survival.

Macroscopy

Grossly difficult to differentiate from ductal carcinoma no special type. Tumor size varies from 2mm to 1.5cm.2 morphologic subtypes- Pure type-stellate nature with radiating arms and central elastosis. Sclerosing type-diffuse ill defined appearance.

Histopathology

This tumor composed predominantly of well formed open tubules, lining by single layer of epithelial cells, enclosing a lumen. Cells are small to medium sized, cuboidal to low columnar and have low grade nuclear features. Elastosis is considered as hallmark of tubular carcinoma. Mitosis is rare. Cellular desmoplastic stroma is characteristic in tubular carcinoma especially at tumour periphery. >90% of the lesion should have tubular morphology to be called as tubular carcinoma.These tumours are ER/PR positive.

Invasive cribriform carcinoma

These are uncommon special type of breast cancer and have excellent prognosis.

Macroscopy

The Tumors are firm ,often have stellate configuration. Size measures from 1 - 3cm in diameter

Histopathology

Tumour shows a sieve like pattern of growth. Tumours consists of round, angulated masses and island of small cells in a reactive desmoplastic stroma. Well- defined punched out spaces filled with mucin are seen.

MUCINOUS CARCINOMA

Macroscopy

They usually form sharply defined tumors ,having soft consistency and have a glistening /gelatinous surface on cutting. Tumor size varies from 1 to 5cm.

Histopathology

Mucinous carcinoma have small islands or clusters of uniformly rounded epithelial cells with an extensive extracellular lake of mucin background. Tumor cells are of small size with minimal cytoplasm and have dark stained nuclei.> 90% of the tumor mass must show mucinous morphology. They have a good prognosis. They are ER/PR positive ,HER2/neu negative. Mucinous carcinoma with neuroendocrine differentiation, tend to have a good prognosis.[36]

MEDULLARY CARCINOMA

Medullary carcinoma is usually occurs in the sixth decade of life. It has a better prognosis although they have high nuclear grade, aneuploidy and absence of hormone receptors expression. There is overexpression of adhesion molecules like E-cadherin which limits metastasis. 13% of cancers arising in BRCA1 carrier are medullary type.

Macroscopy

They are well circumscribed mass, soft in consistency and are measuring 1 to 4 cm in diameter.

Histopathology

Main criteria includes

- Syncitium like sheets of large cells present. Cells have abundant cytoplasm with pleomorphic vesicular nuclei and prominent nucleoli. This pattern should be present at least 75% of tumor. Glands and tubules are not seen.
- Scanty stroma with moderate to severe lymphoplasmacytic infiltrate
- Pushing borders.

Invasive papillary carcinoma

This is a rare type of breast cancer and occurs frequently in elderly women and men as well.

Macroscopy

Grossly they have varied appearance, well demarcated, soft consistency.Size ranges from 1 - 3cm in diameter.

Histopathology

Papillary structures with fibrovascular core is characteristic feature of tumor. Most of the tumours are bounded by fibrosis , chronic inflammation and hemorrhage. Varied morphologic appearances. Mitosis seems to be increased.

Invasive micropapillary carcinoma

It is an uncommon type, the epithelial cells form micropapillae with out fibrovascular core. It presents as solid tumor and has high lymph node metastasis. Tumor cells have moderate pleomorphism and low mitotic activity.

MIXED TYPE

Mixed ductal and lobular carcinoma

In this tumor ductal component accounts for 10 to 90% of the tumor. Incidence varied from 2 to 6% of all breast tumors.

Mixed ductal and special type of tumor

In this tumor, special type of tumor may be tubular, invasive cribriform or mucinous. Special type of tumor should form more than 10% of tumor mass.

Rare types

Rare types of breast tumor includes

1. Secretory carcinoma
2. Apocrine carcinoma
3. Neuroendocrine carcinoma

Secretory carcinoma

It occurs in all ages. They are well defined and firm neoplasm. They are less than 3cm in diameter. Microscopically they have well defined border. It

is a low grade tumor with solid, microcystic and tubular pattern. The characteristic feature is intracellular and extracellular vacuoles which may contain mucinous material. Prognosis is excellent in children but in adults it is not so good.[37]

Apocrine carcinoma

Carcinomas showing cytological and immunological features of apocrine cells in more than 90% of tumor are called as apocrine carcinoma. They account for less than 1% of the breast tumors. Neoplastic cells have abundant, granular, eosinophilic cytoplasm with high grade nuclear features. They usually negative for ER, PR but frequently positive for androgen receptor and GCDFP-15.

Neuroendocrine carcinoma

Primary neuroendocrine tumour of the breast will express neuro endocrine markers in more than 50% of the cells. 1 to 4% of the tumors are neuroendocrine tumors. Histologically they include solid, small cell and large cell neuro endocrine tumors. Solid type will have nest and trabeculae separated by thin fibrovascular stroma. They may have rosette like structure and peripheral palisadding. They tend to have poor prognosis than conventional invasive ductal carcinoma breast.

Metaplastic carcinoma

These are heterogeneous group of tumors composed of malignant epithelial elements and mesenchymal elements. Squamous and/or spindle cell components are present. Mesenchymal elements includes cartilage, bone and myxoid stroma. These tumors are uncommon. Grossly they form large, firm, well defined tumor measuring up to 5cm in diameter. Microscopically two major subtypes include monophasic and biphasic. These tumors are triple negative and express cytokeratin 5/6, cytokeratin 14, cytokeratin 7 and CAM5.2.

Investigation

1. Mammography

2. Cytology

3. Needle core biopsy

4. Open biopsy and frozen section

Mammography

Mammography helps in detecting small tumors of size 1mm to 2mm. Mammography detects calcified lesions. Cancer breast is associated with 50%to 60% of calcifications.

Cytology

All palpable breast lesions are initially investigated with fine needle aspiration cytology. Although it is a cost effective and simple method, invasion cannot be detected. Role of FNAC in cancer breast. The confirmation of clinically suspected and inoperable cancer. To investigate the suspected recurrence or metastasis in case of previously diagnosed cancer. To obtain tumor cells for special analysis such as IHC and cytogenetic analysis.

Needle core biopsy

Needle core biopsy is useful in diagnosing cancer breast with invasive component. Hormonal status(ER,PR) and HER2 overexpression can be studied.

Open biopsy and frozen section

There are two types of biopsy one is excision biopsy and the other one is incisional biopsy. Frozen sections are used to evaluate margin and to confirm the diagnosis of suspected invasive cancer detected by other methods. Disadvantages of frozen section are Sampling error, Technical error and Histological misinterpretation

Staging

TNM system of staging is commonly used and it is adopted by American joint committee on cancer(AJCC).

Grading

Nottingham modification of the Bloom-Richardson system of grading is commonly used. It is based on microscopic assessment of tubule formation, nuclear pleomorphism and mitotic count.

Prognostic and predictive factors

Prognosis of breast cancer varies widely. Prognosis mainly determined by pathological examination of primary cancer and axillary node. It is useful for patient counselling, appropriate treatment and clinical trials.

Invasive versus in situ carcinoma

Most of the in situ carcinoma can be cured adequate where as more than 50% patients with invasive cancer have metastasis.[38,39,40.]

Distant metastasis

Distant metastasis is a poor prognostic factor. But it depends upon other factors like tumor type and location metastasis.

Lymph node metastasis

In the absence of distant metastasis axillary nodal involvement is the important factor. Metastatic foci of less than 0.2cm are called as micrometastasis. Macrometastasis are more than 0.2cm in size and are important

prognostic factor. Nearly 10% - 20% of women without axillary node metastasis may have recurrence due to metastasis via blood or internal mammary node.

Tumor size

Size of the tumor is second most important prognostic indicator. Number of axillary nodal metastasis is increases with the size of the tumor.

Locally advanced disease

Cancer involving chest wall and skin are difficult to treat. They are associated with poor prognosis.

Inflammatory carcinoma

Carcinoma breast presenting with swelling and skin involvement have poor prognosis.

Histologic subtype

When compared to carcinoma of breast no special type, women with special type of invasive cancer (tubular, medullary, mucinous, lobular) have increased survival rate.

Histologic grade

The Nottingham modification of Bloom Richardson Histologic Score is the commonly used grading system. It combines nuclear grade, mitotic rate and tubule formation to classify carcinoma of breast as Grade I, II and III carcinoma.[41]

Estrogen and progesterone receptors

Patients with ER and PR positive breast cancer have increased disease free survival rate. ER and PR positivity is positively correlated with response to hormonal therapy.

HER2/neu

Response to trastuzumab is predicted with overexpression of HER2/neu oncogene. Women with tumor which showing overexpression will have poor prognosis. Her2/neu overexpression is correlated with high grade tumor.[42]

Types of margin

Better prognosis has been observed in tumors with pushing type of margin. This applies to medullary carcinoma as well as other well circumscribed tumors.

Micro vessel density

Invasive breast carcinoma will behave in aggressive manner when they have increased vascularity in the surrounding stroma.

Elastosis

Tumors with no elastosis will have reduced response to hormonal therapy.

TREATMENT

Type and extent of the breast cancer determines the treatment .

- Surgery
- Radiation therapy
- Hormonal therapy
- Chemotherapy
- Target therapy

Simple mastectomy, radical mastectomy and modified radical mastectomy are the some of the surgical methods.[43] Radiation therapy is used in postoperative period and to control the local recurrence of carcinoma. The chemotherapy is given as an neoadjuvant therapy and after local treatment and also given in breast cancers with axillary node metastasis. Chemotherapy can be used in combination with surgery and radiation in patients with large (>3 cm) tumors in order to avoid mastectomy. The hormonal therapy is used in hormone receptor-positive breast carcinomas.

MATERIALS AND METHODS

STUDY DESIGN:

The present study is a Prospective study conducted in the Department of Pathology during the period from July 2016 to June 2017. Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

A total sample of 30 cases of invasive ductal carcinoma breast were analyzed.

PLACE OF STUDY:

Department of Pathology, Coimbatore Medical College, Coimbatore

STUDY PERIOD:

July 2016 – June 2017

INCLUSION CRITERIA:

- Invasive ductal carcinoma of breast no specific type.
- Other special histologic subtypes

EXCLUSION CRITERIA:

1. Male patients
2. Ill fixed specimen
3. Patients with other malignancies.

The study was done in 30 invasive ductal carcinoma of breast cases and some special subtypes of breast cancer sent from Department of Surgery and Department of Surgical Oncology, Coimbatore Medical College Hospital, Coimbatore for histopathological examination .

Hematoxylin and Eosin stained microscopic slides of the primary tumor were reviewed to confirm the diagnosis, to define tumor subtype and to standardize grading of invasive ductal carcinoma according to the Nottingham Modification of the Bloom and Richardson system.

GRADE	**SCORE**
I	3T0 5
II	6&7
III	8&9

Microscopic grading of carcinoma of breast: **Nottingham Modification of the Bloom and Richardson system**
Tubule formation 1 point: Tubular formations in >75% of the tumor 2 point: Tubular formations in 10-75% of the tumor 3 point: Tubular formations in <10% of the tumor
Nuclear pleomorphism 1 point: Nuclei with minimal variation in size and shape 2 point: Nuclei with moderate variation in size and shape 3 point: Nuclei with marked variation in size and shape
Mitotic count 1 point: upto 11/10 hpf, 2 point: 12 to 23/10 hpf, 3 point: 23 or more/ 10 hpf

ALLRED/QUICK SCORE SYSTEM

Score	Score for proportion	Score for intensity
0	No staining	No staining
1	<1% Nuclei staining	Weak staining
2	1%-10% Nuclei staining	Moderate staining
3	11%-33% Nuclei staining	Strong staining
4	34%-66% Nuclei staining	Strong staining
5	67%-100% Nuclei staining	Strong staining

ER and PR markers were considered positive when the combined score for proportion and intensity is 3 or more.

HER2 SCORING WAS DONE ACCORDING TO THE FOLLOWING TABLE

STAINING PATTERN	**SCORE**	**HER2/neu Overexpression**
No staining or membrane staining<10% tumor cells	0	Negative
Faint/perceptible membrane staining in >10% cells	1+	Negative
Weak to moderate complete membrane staining in >10% cells	2+	Weak
Strong complete membrane staining in >30% cells	3+	Strong

CYTOPLASMIC BCL2 EXPRESSION WAS SCORED ACCORDING TO THE FOLLOWING TABLE

BCL2 EXPRESSION-SCORE	PERCENTAGE OF CELLS
NEGATIVE SCORE 0	No Staining
POSITIVE SCORE 1+	Slight staining in some or most of the cells
POSITIVE SCORE 2+	Moderately strong staining
POSITIVE SCORE 3+	Strong staining in almost all cells

Formalin fixed and paraffin embedded tissue specimen of invasive breast carcinoma were examined. Four micron sections were cut and stained for immunohistochemistry with mouse monoclonal antibodies.[70] The staining was done as per the following protocol.

REAGENTS USED IN IMMUNOHISTOCHEMISTRY

1.Peroxide block

2. Power block

3. Chromogen - Diaminobenzidine

4. Liquid DAB substrate

5. Super enhancer

6. Poly HRP reagent

7. Hematoxylin- counter stain

8. Buffer solutions

BUFFERS USED

1. TRIS EDTA : pH- 9.0

RIS buffer salt : 6.05 gm

DisodiumEDTA : 0.744gm

Distilled water : 1000ml

2. TRIS BUFFER SALINE (pH – 8)

TRIS buffer salt : 6.05gm

Sodium chloride : 8gm

Distilled water : 000ml 1N

Hydrochloric acid : 3.5-4ml

3. CITRATE BUFFER pH-6

Trisodium citrate : 2.94 gm

Distilled water : 1000ml

1N Hydrochloric acid : 4 ml

IMMUNOHISTOCHEMISTRY PROCEDURE

1. Overnight incubation of slides at 60^{0}c.
2. 30 minutes(60-70^{0}c)first incubator.put in xylene immediately.
3. Xylene three changes,each ten minutes.
4. Absolute alcohol two changes,each 5 minutes.
5. Running tap water-5 minutes(meanwhile preheat antigen retrieval buffer (600^{0} c -3 minutes)

6. Antigen retrieval

   450^0 c-5min-three times

   800^0 c-5 minutes-three times.add distilled water inbetween if buffer quantity appears decreased.cool to room temperature.

7. Distilled water-5 minutes
8. Peroxide block-10 minutes
9. TBS wash-two times- 5 minutes each
10. Primary antibody(bcl2)-one hour
11. TBS wash-three times-one immediate wash,other two 5 minutes each
12. DAB-ab-5 to 7 minutes(1ml of buffer+1 drop chromogen)
13. Distilled water(just rinse)
14. Dip in haematoxylin
15. Blueing-5 minutes
16. Dry
17. Mount

Statistical analysis:

The collected data was tabulated and analyzed. Continuous data was Expressed as mean. Statistical correlation between expression of Bcl2 and

histopathological grade, ER, PR and Her2/neu expression were performed as per Chi square test. P values of less than 0.05 were considered as significant.

OBSERVATION AND RESULTS

TABLE 1: AGE incidence of Breast Carcinoma

AGE	NUMBER OF CASES	PERCENTAGE (%)
<40 YEARS	8	26.7
40-50 YEARS	5	16.7
50-60 YEARS	10	33.3
>60 YEARS	7	23.3
TOTAL	30	100.0

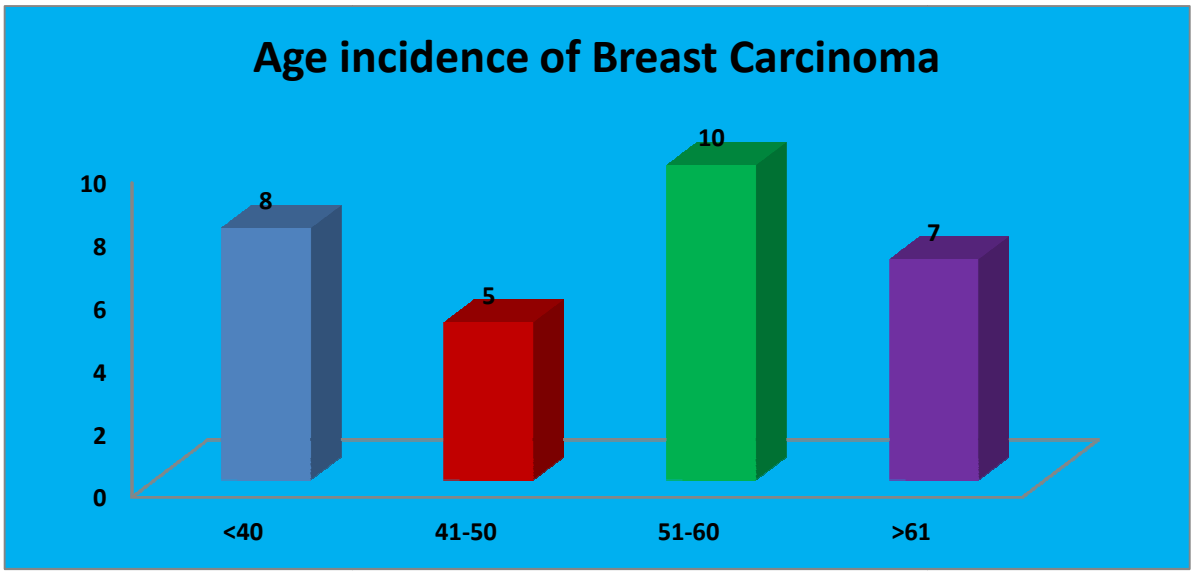
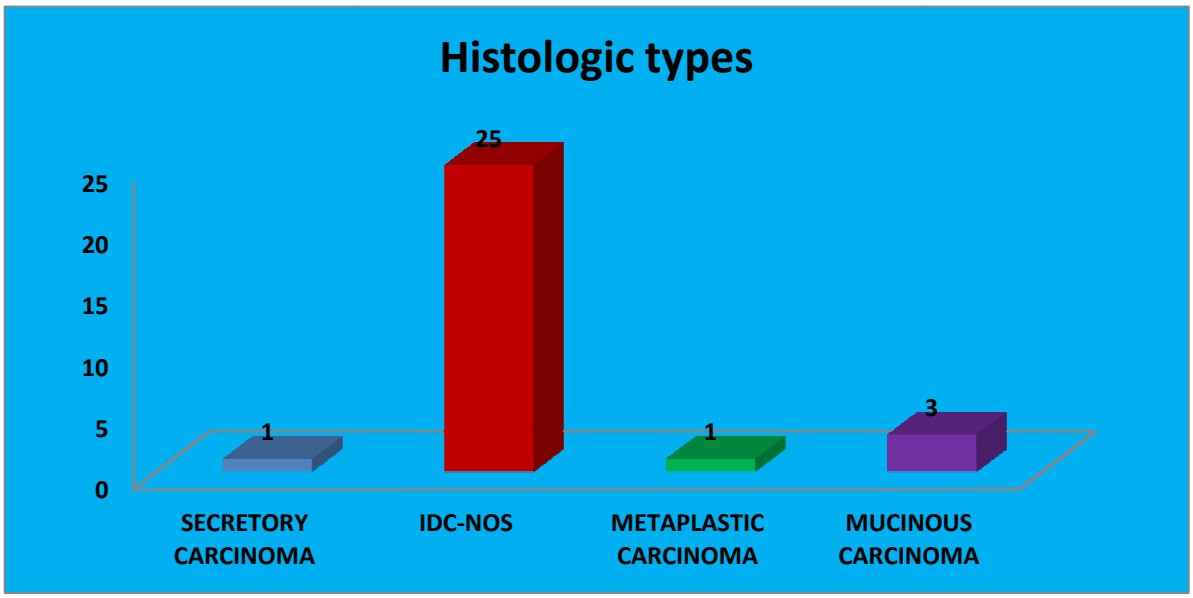


Table 2: Distribution of Histological variants in Breast Carcinoma

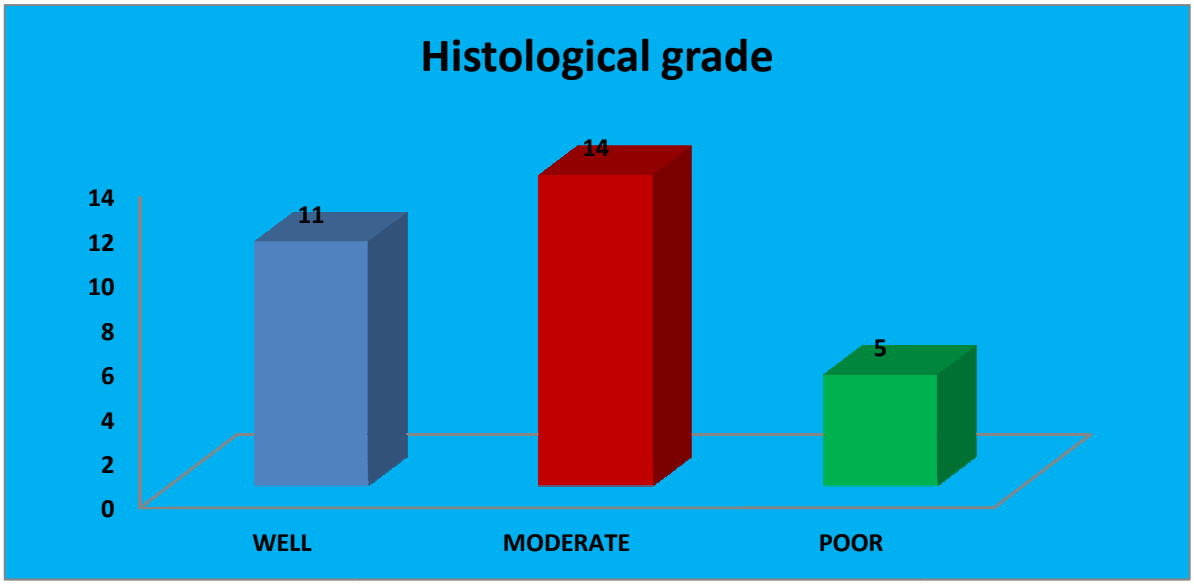
HISTOLOGIC TYPES	NUMBER OF CASES	PERCENTAGE (%)
SECRETORY CARCINOMA	1	3.3
IDC-NOS	25	83.3
METAPLASTIC CARCINOMA	1	3.3
MUCINOUS CARCINOMA	3	10.0
TOTAL	30	100.0



Out of 30 cases we have taken ,25 cases are IDC-NOS type,3 cases are Mucinous carcinoma,1Medullary carcinoma,1Metaplastic carcinoma.All these cases are randomly selected.

Table 3: Distribution of Histological grade in Breast Carcinoma

GRADE	NUMBER OF CASES	PERCENTAGE (%)
1(WELL)	11	36.7
2(MODERATE)	14	46.7
3(POOR)	5	16.7
TOTAL	30	100.0



Among 30 cases,11 belong to Well differentiated histologic grade(36.7%) , 14 moderately differentiated grade(46.7%) and 5 Poorly differentiated grade tumours(16.7%) are included.

Table 4: Distribution of IDC of breast according to different age group

AGE	NUMBER	Percentage (%)
31-40	8	32.0
41-50	4	16.0
51-60	8	32.0
>60	5	20.0
TOTAL	**25**	**100%**

Total of 25 cases were studied and the following observations were obtained.

The age of the patients ranges from 35 to 76 years with mean age of 52 years.

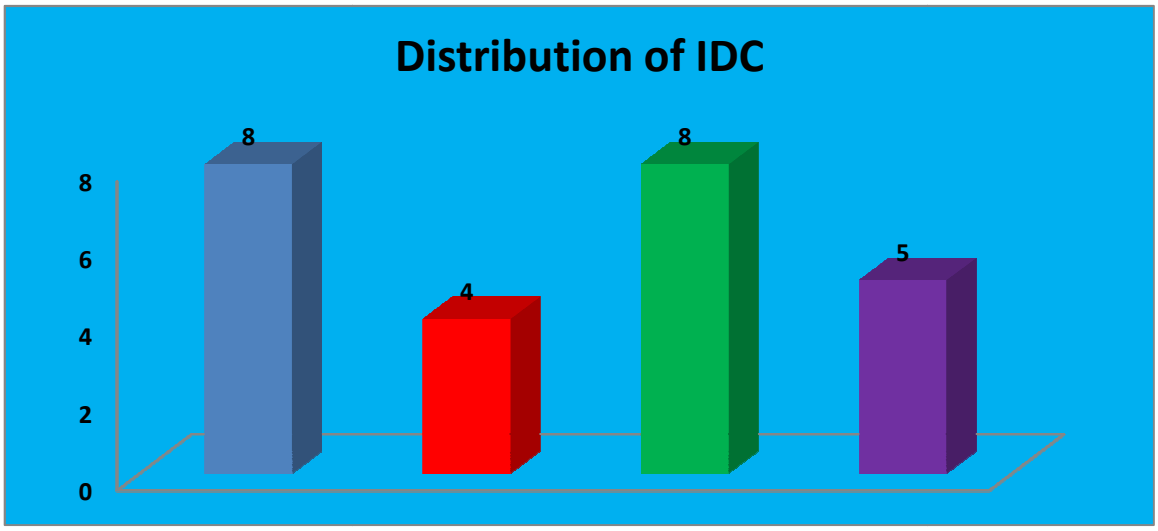


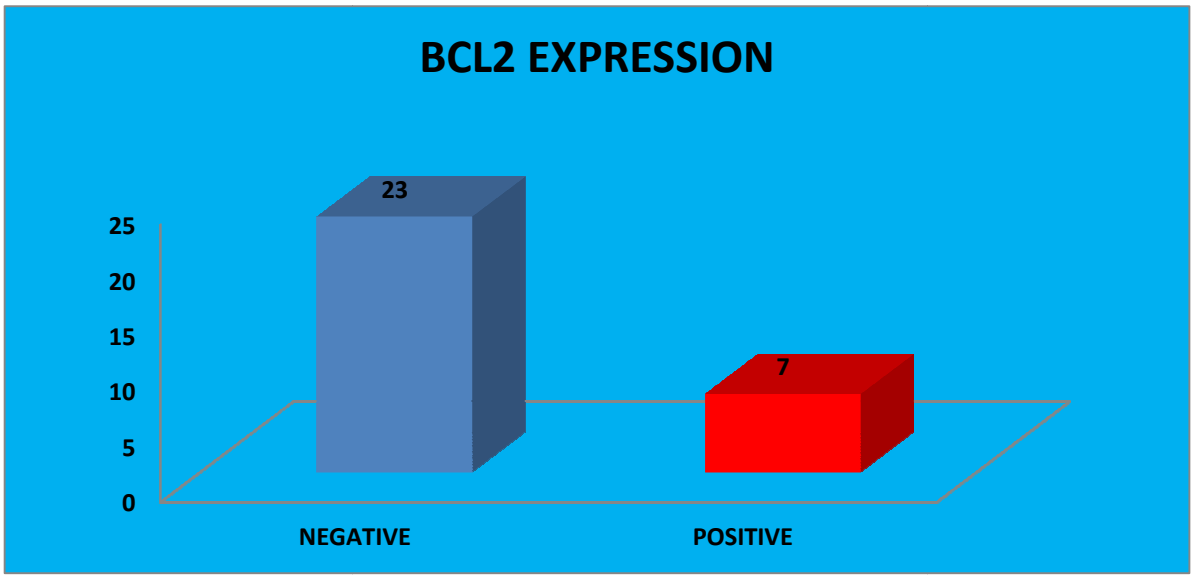
Table 5:MEAN AGE OF GROUP

	N	MINIMUM	MAXIMUM	MEAN	STANDARD DEVIATION(SD)
AGE	30	35	76	52.70	11.95

Mean age of the study group is 52.70 years Lowest age of this study is 35 years

TABLE 6: BCL2 EXPRESSION IN BREAST CARCINOMA

BCL2 EXP	NUMBER OF CASES	PERCENTAGE (%)
NEGATIVE	23	76.7
POSITIVE	7	23.3
TOTAL	30	100.0



Out of 30 cases we have taken,7 cases(23.3%) shows intense grade(+++) III positivity,23 cases (76.7%) shows no expression. Staining for Bcl-2 classified is into four groups:

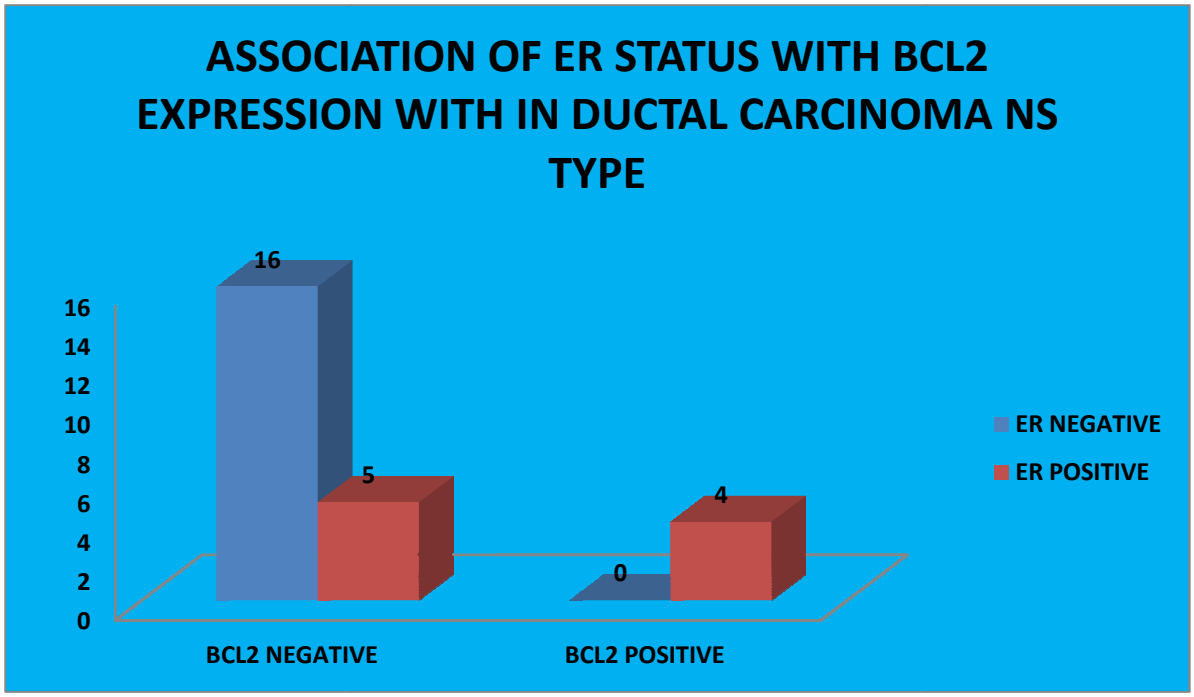
- No staining present in any of the breast cancer cells (-),
- Slight staining in some cells or in most of the cells (+),
- Moderately strong staining(++), or

Strong staining present in almost all cells (+++). Classification was done by a seniorpathologist

Table 7: ASSOCIATION OF ER STATUS WITH BCL2 EXPRESSION WITH IN DUCTAL CARCINOMA NST TYPE

ER	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
NEGATIVE	16(100.0%)	0(0.0%)	.004*
POSITIVE	5(55.6%)	4(44.4%)	

*-statistically significant (P<0.05)

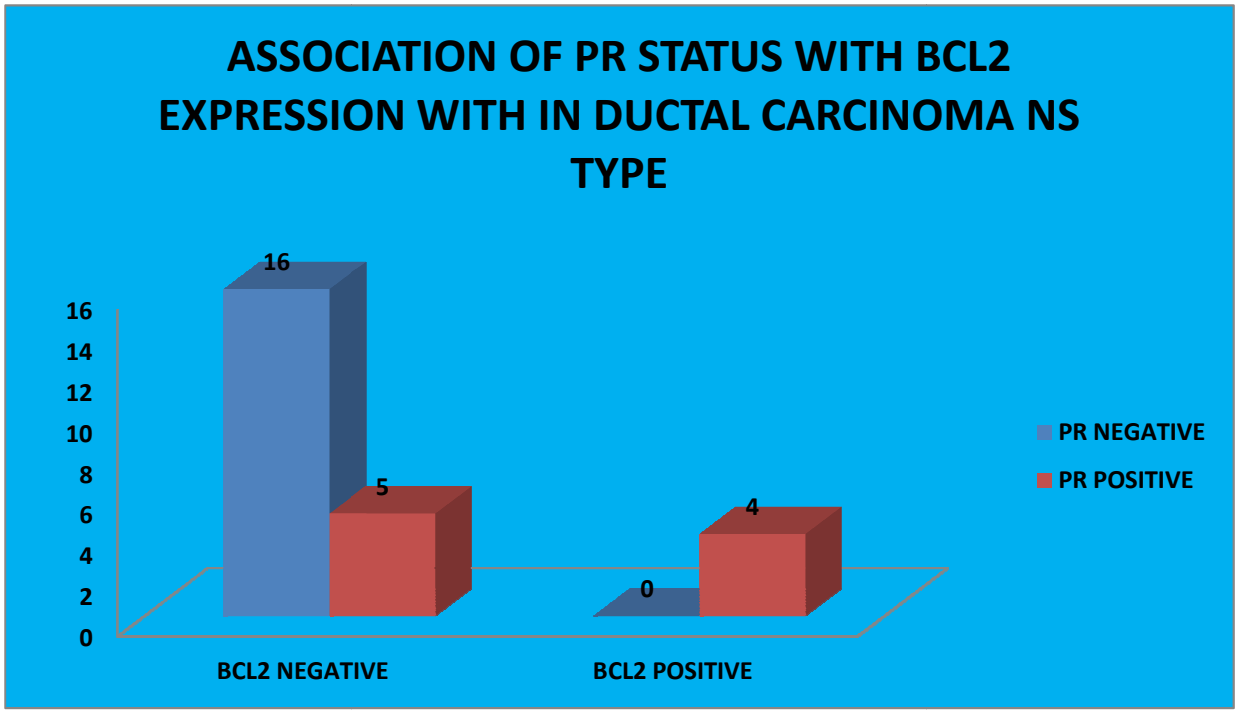


In ductal carcinoma NS type,44.4% of ER positive cases show Bcl2 positivity,which is statistically significant(p value-0.004)

Table 8: ASSOCIATION OF PR STATUS WITH BCL2 EXPRESSION WITH IN DUCTAL CARCINOMA NS TYPE

PR	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
NEGATIVE	16(100.0%)	0(0.0%)	.004*
POSITIVE	5(55.6%)	4(44.4%)	

*-statistically significant (P<0.05)



4 out of 9 cases(44.4%) of PR+ ductal carcinoma NS type,shows Bcl2 positivity with p value 0.004(statiscally significant p value<0.05)

TABLE 9: ASSOCIATION OF HER2/NEU WITH BCL2 EXPRESSION WITH IN DUCTAL CARCINOMA NS TYPE

HER2/NEU	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
NEGATIVE	17(81.0%)	4(19.0%)	.341
POSITIVE	4(100.0%)	0(0.0%)	

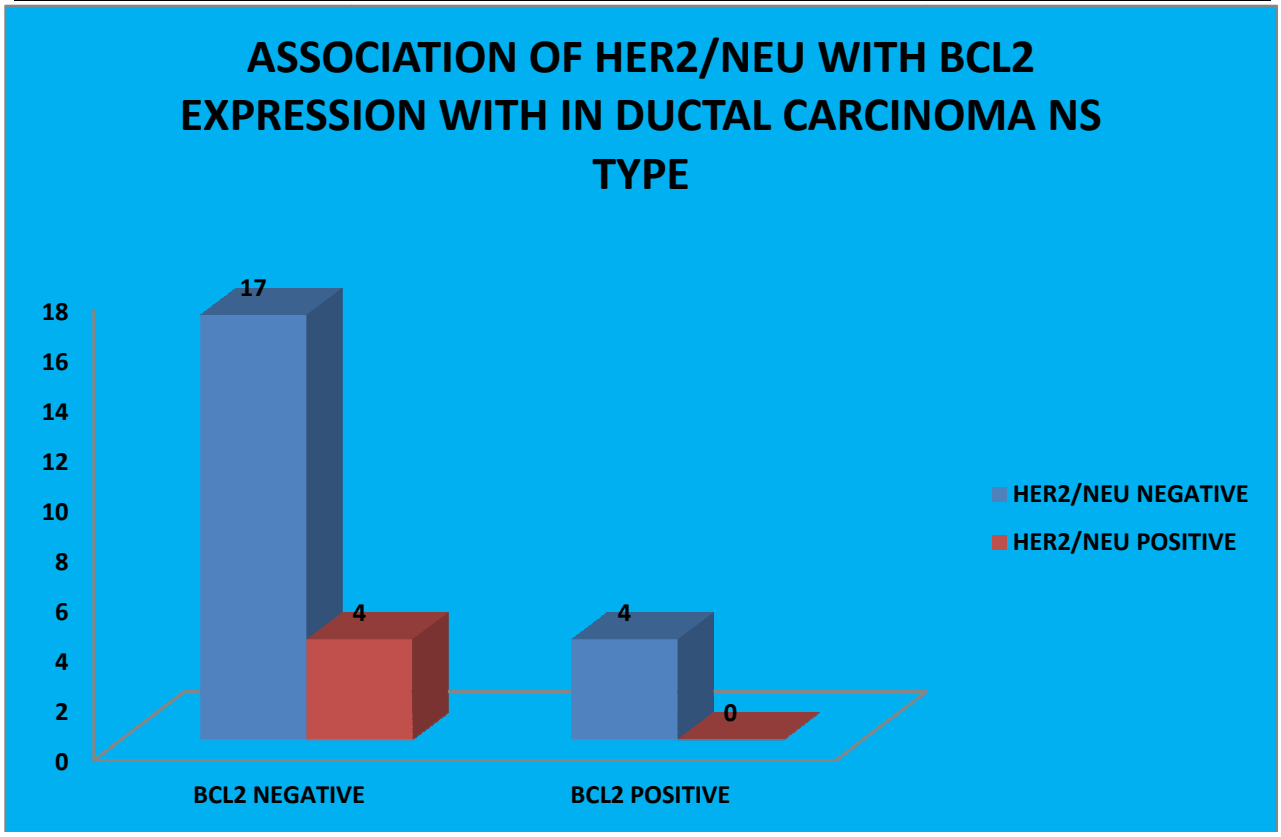


TABLE 10: ASSOCIATION OF TUMOUR SIZE WITH BCL2 EXPRESSION WITH IN DUCTAL CARCINOMA NS TYPE

TUMOUR SIZE	BCL2 EXPRESSION		P VALUE
	NEGATIVE	POSITIVE	
T1	1(33.3%)	2(66.7%)	.024*
T2	11(84.6%)	2(15.4%)	
T3	9(100.0%)	0(0.0%)	

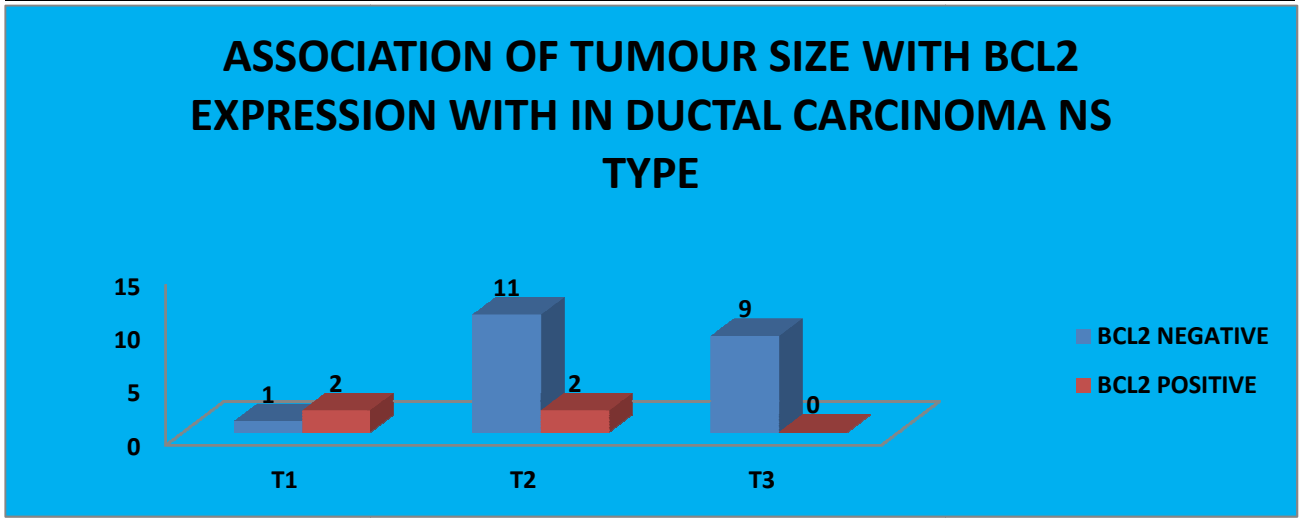


TABLE 11: ASSOCIATION OF LYMPH NODE STATUS WITH BCL2EXPRESSION WITH IN DUCTAL CARCINOMA NS TYPE

LYMPH NODE STATUS	BCL2 EXPRESSION		P VALUE
	NEGATIVE	POSITIVE	
N0	4(80.0%)	1(20.0%)	.680
N1	6(85.7%)	1(14.3%)	
N2	6(75.0%)	2(25.0%)	
N3	5(100.0%)	0(0.0%)	

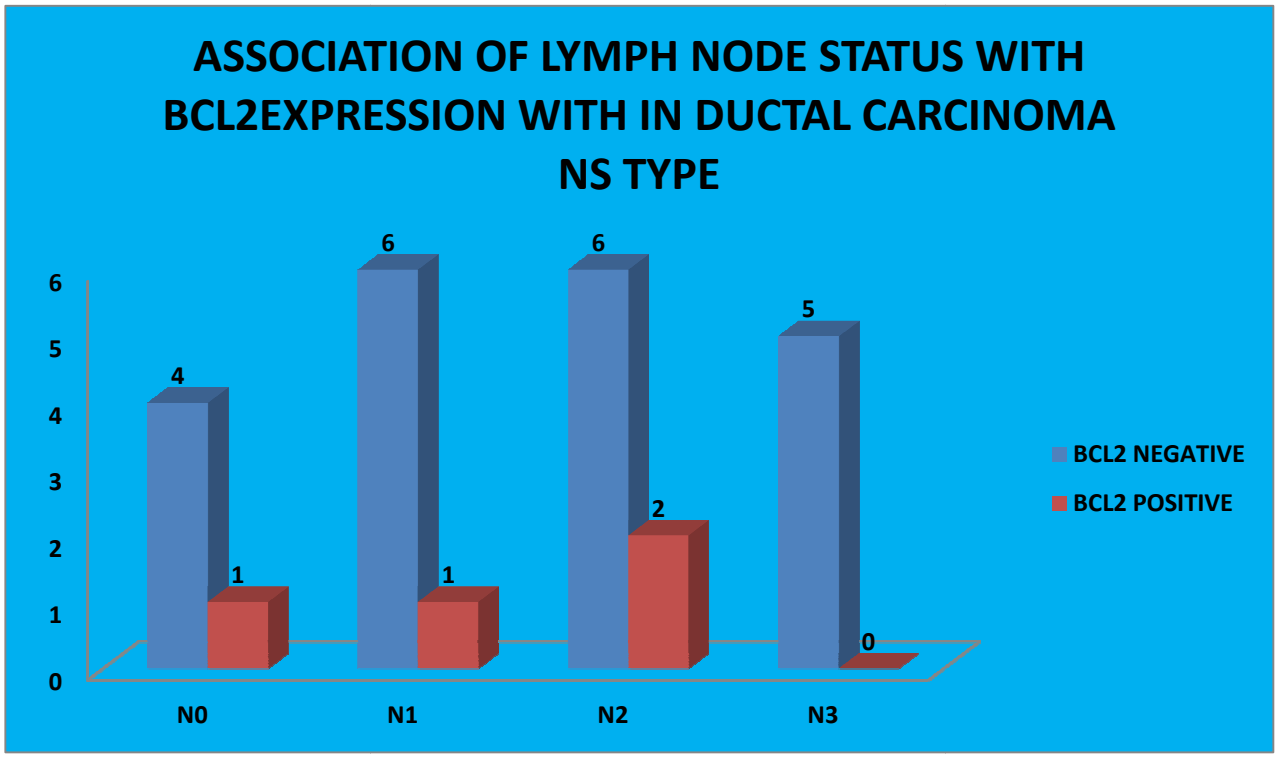


TABLE 12: ASSOCIATION OF GRADE STATUS WITHBCL2 EXPRESSION WITH IN DUCTAL CARCINOMA NS TYPE

GRADE	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
I	6(75.0%)	2(25.0%)	.582
II	12(85.7%)	2(14.3%)	
III	3(100.0%)	0(0.0%)	

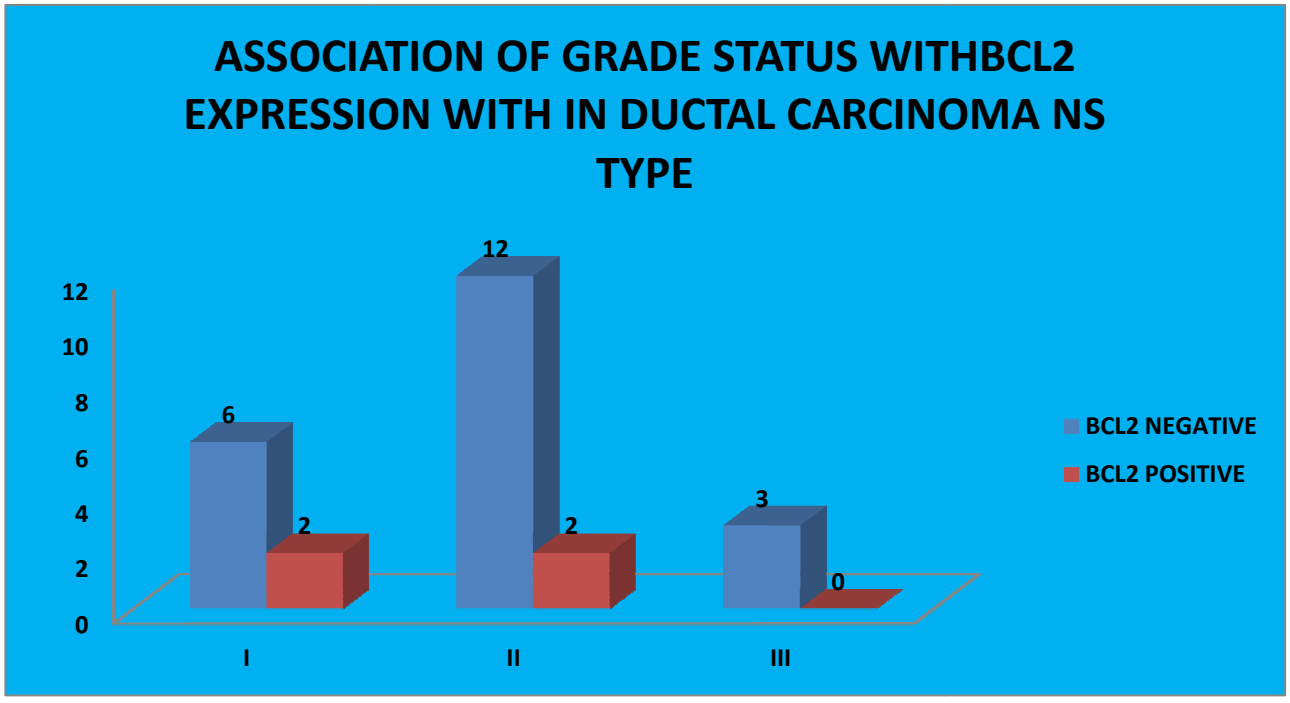


Table 13: BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

Special type	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
Mucinous ca	0(0.0%)	3(100.0%)	.025*
Metaplastic &secretory ca	2(100.0%)	0(0.0%)	

*-statistically significant (P<0.05)

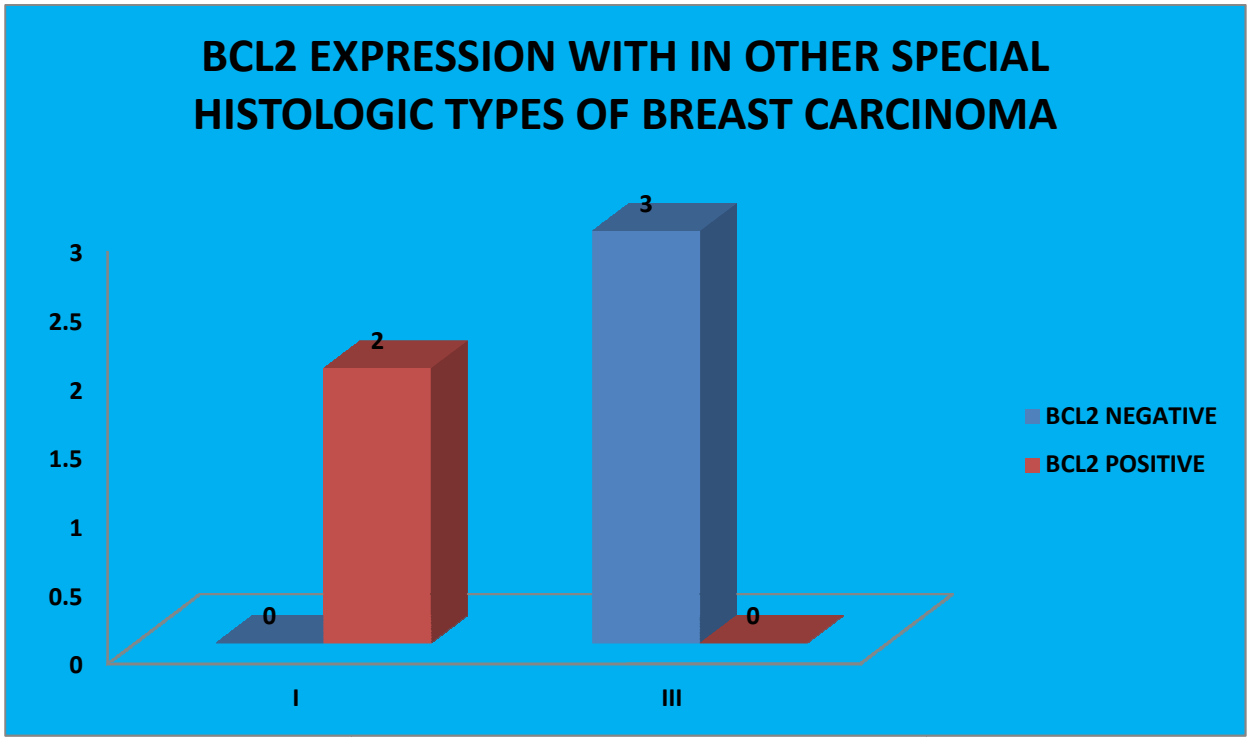


Table 14: ASSOCIATION OF ER STATUS WITH BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

ER	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
NEGATIVE	2(100.0%)	0(0.0%)	.004*
POSITIVE	0(0.0%)	3(100.0%)	

*-statistically significant (P<0.05)

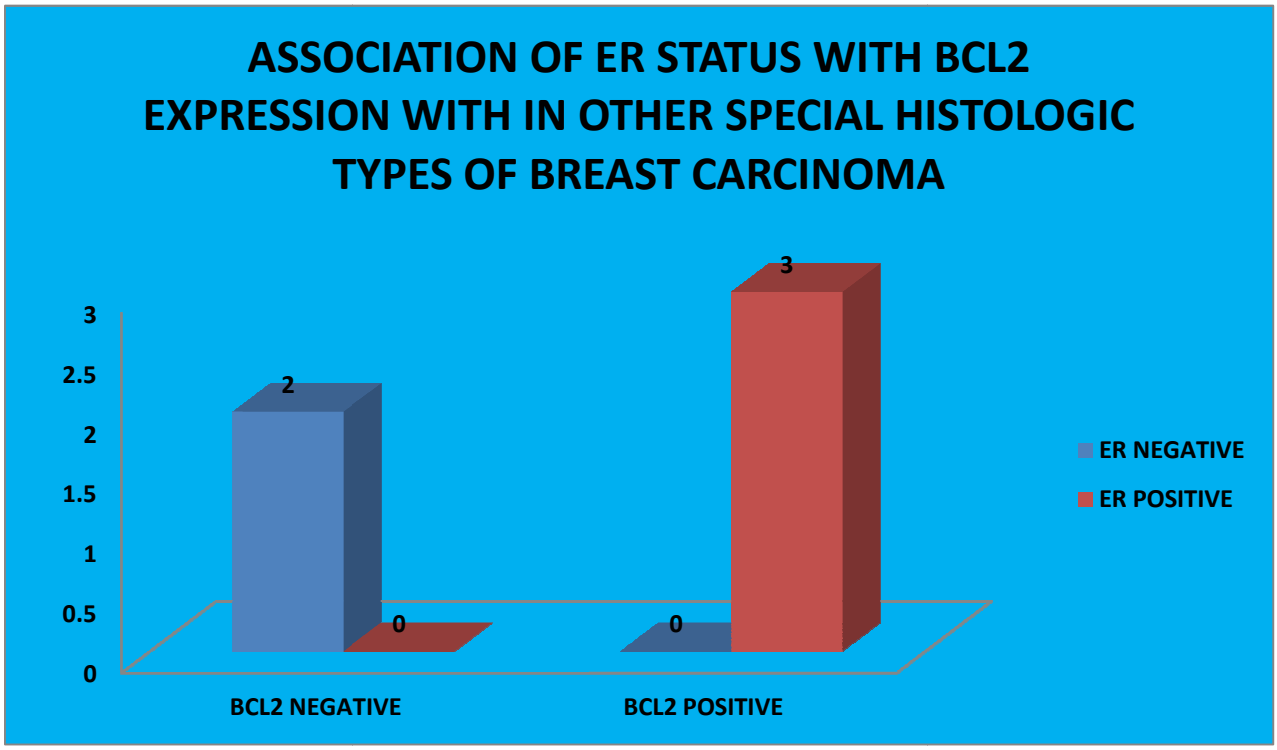


Table 15: ASSOCIATION OF PR STATUS WITH BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

PR	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
NEGATIVE	2(100.0%)	0(0.0%)	.004*
POSITIVE	0(0.0%)	3(100.0%)	

*-statistically significant (P<0.05)

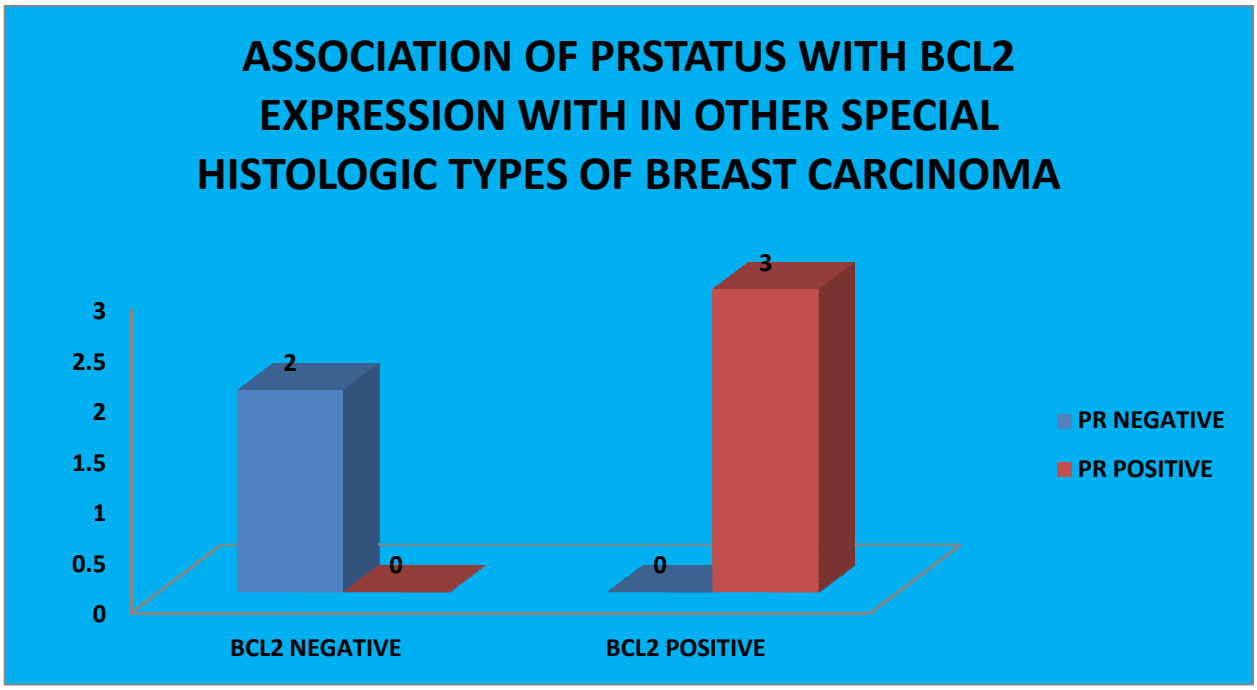


Table16: ASSOCIATION OF HER2 STATUS WITH BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

HER2 status	BCL2 EXPRESSION	
	NEGATIVE	POSITIVE
NEGATIVE	2(40.0%)	3(60.0%)

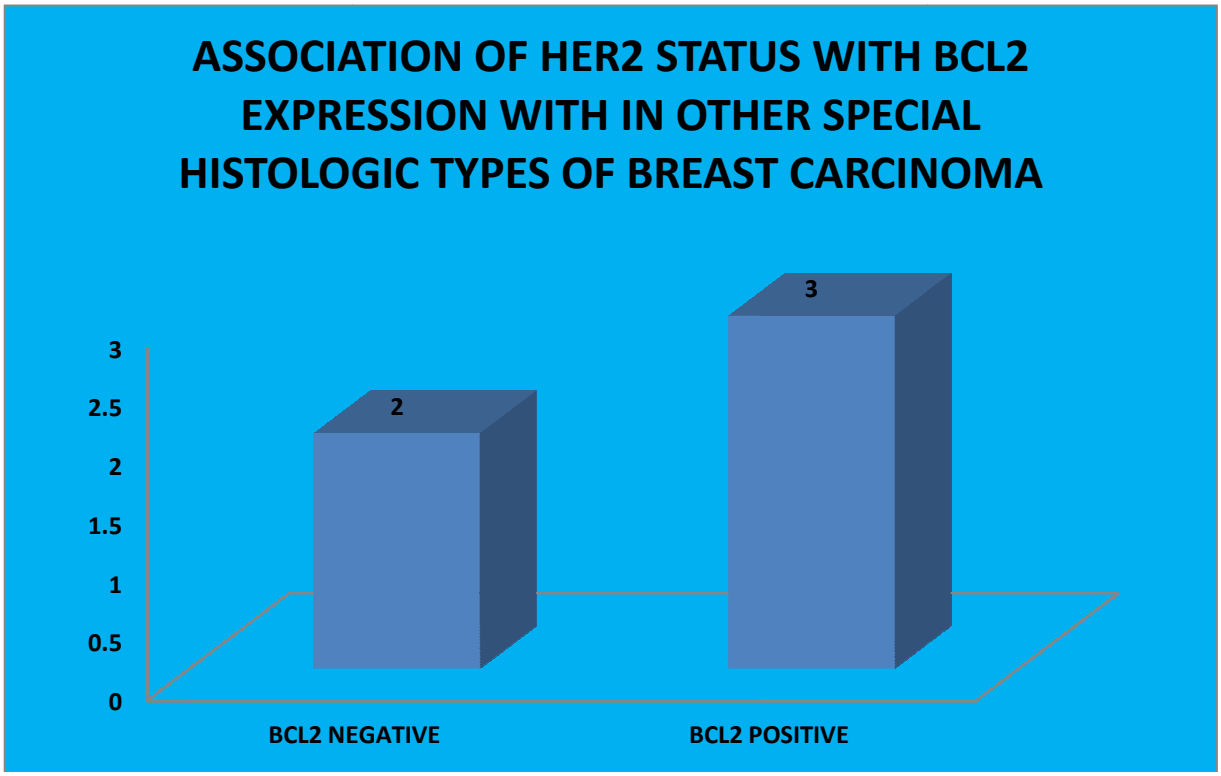


Table 17: ASSOCIATION OF LYMPHNODE STATUS WITH BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

LYMPHNODE STATUS	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
N0	2(50.0%)	2(50.0%)	.361
N1	0(0.0%)	1(100.0%)	

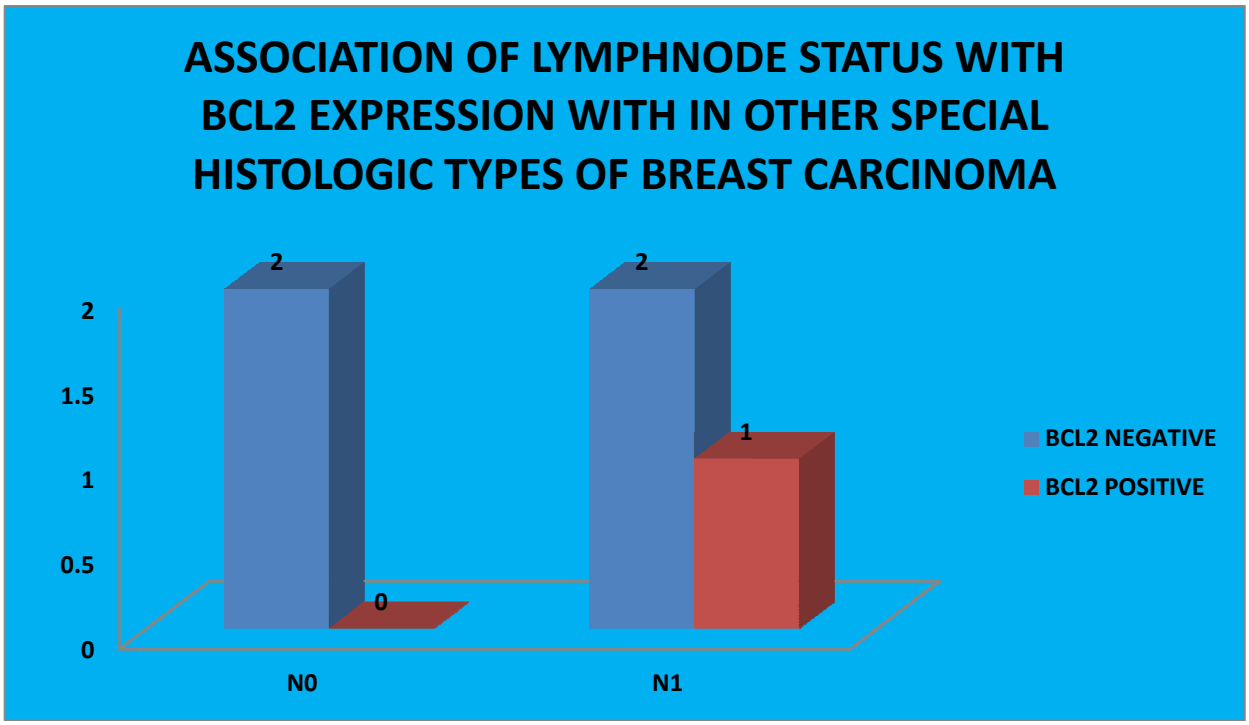


Table 18: ASSOCIATION OF TUMOUR SIZE WITH BCL2 EXPRESSION WITH IN OTHER SPECIAL HISTOLOGIC TYPES OF BREAST CARCINOMA

TUMOUR SIZE	BCL2 EXPRESSION		P value
	NEGATIVE	POSITIVE	
T1	1(100.0%)	0(0.0%)	.233
T2	1(50.0%)	1(50.0%)	
T3	0(0.0%)	2(100.0%)	

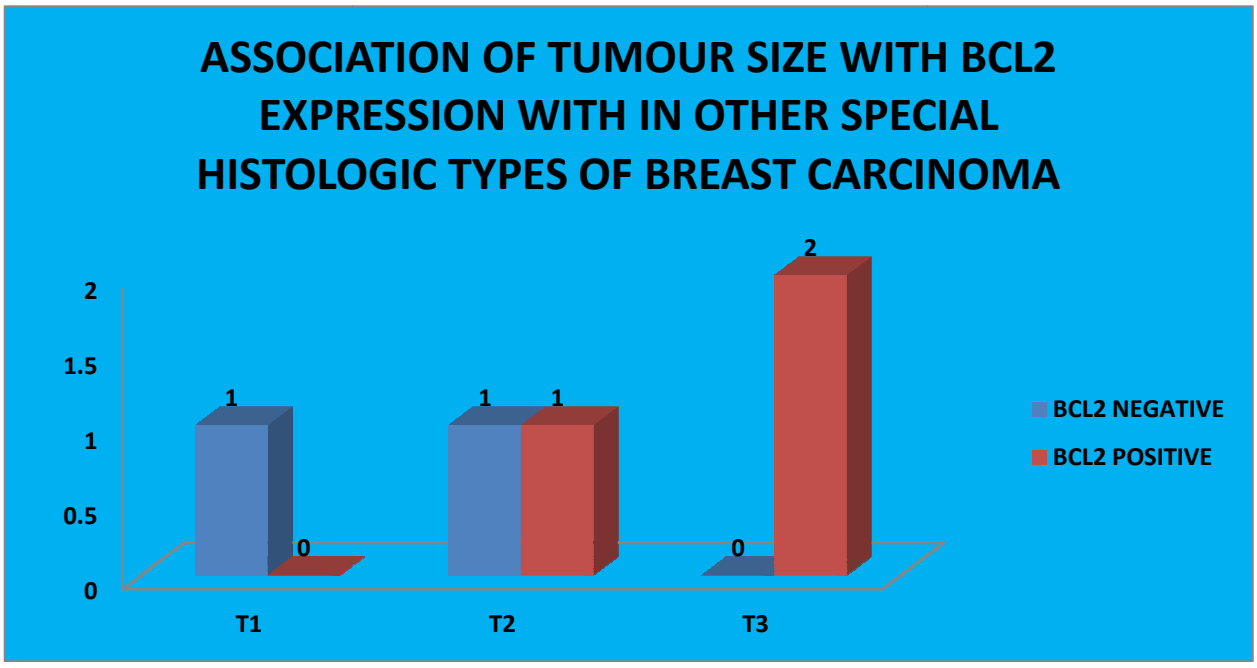
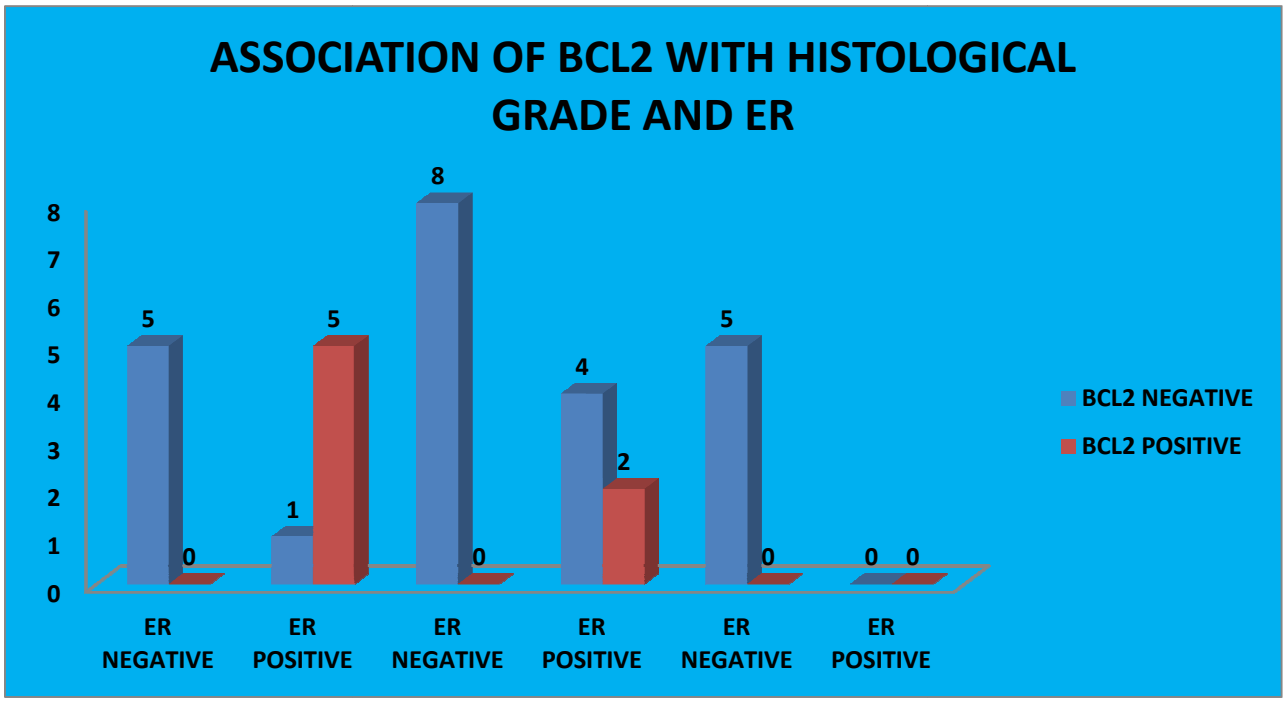


TABLE 19: ASSOCIATION OF BCL2 WITH HISTOLOGICAL GRADE AND ER

GRADE	ER STATUS		BCL2 EXPRESSION		P VALUE
			NEGATIVE	POSITIVE	
GRADE I	ER	NEGATIVE	5(100.0%)	0(0.0%)	.006*
		POSITIVE	1(16.7%)	5(83.3%)	
GRADE II	ER	NEGATIVE	8(100.0%)	0(0.0%)	.075
		POSITIVE	4(66.7%)	2(33.3%)	
GRADE III	ER	NEGATIVE	5(100.0%)	-	-
		POSITIVE	-	-	

*-statistically significant (P<0.05)



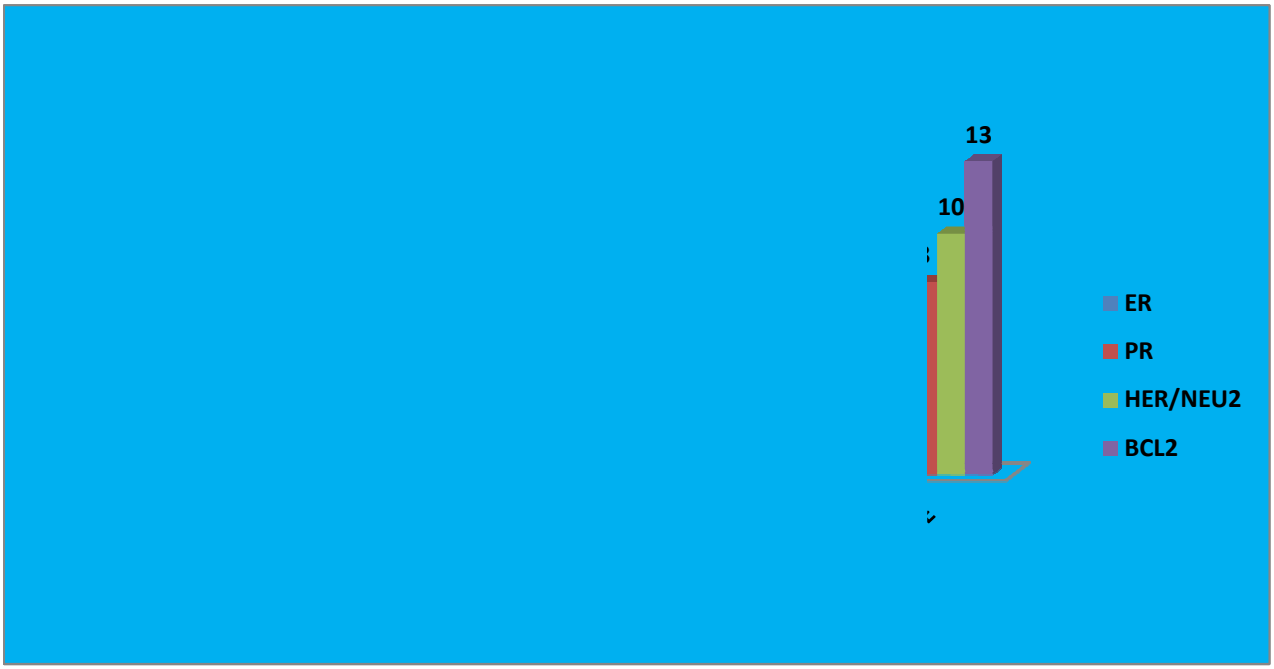
GRADE I GRADE II GRADE III

TABLE 20:IMMUNOHISTOCHEMICAL MARKER EXPRESSION WITH CORRESPONDING NPI GROUPS

MARKER	NPI1		NPI2		NPI3	
	+ VE	-VE	+ VE	-VE	+ VE	-VE
ER	7(70.0%)	3(30.0%)	1(16.7%)	5(83.3%)	4(28.6%)	10(71.4%)
PR	5(5.0%)	5(50.0%)	1(16.7%)	5(83.3%)	6(42.9%)	8(57.1%)
HER/NEU2	-	10(100.0%)	-	6(100.0%)	4(28.6%)	10(71.4%)
BCL EXPRESSION	5(5.0%)	5(50.0%)	1(16.7%)	5(83.3%)	1(7.1%)	13(92.9%)

P VALUE CANNOT BE CALCULATED

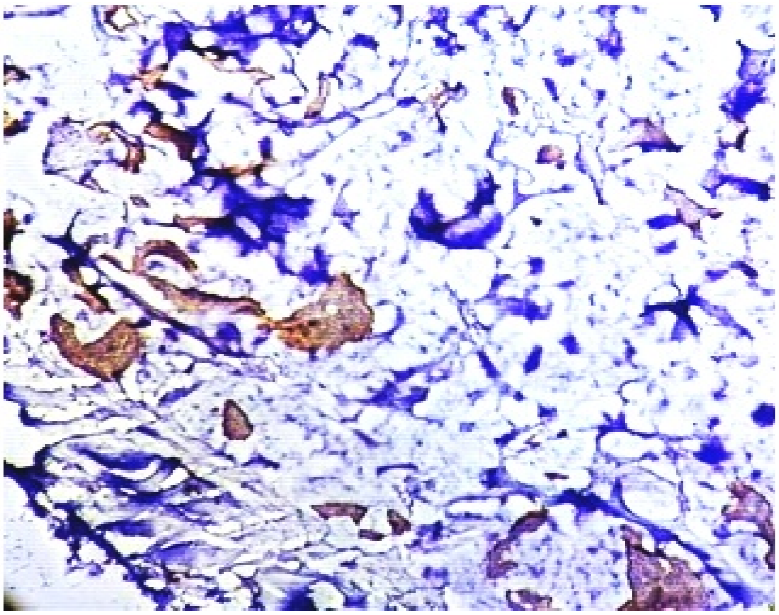
ER,PR,HER2,BCL2 AND NPI GROUPS



NPI 1 **NPI2** **NPI3**

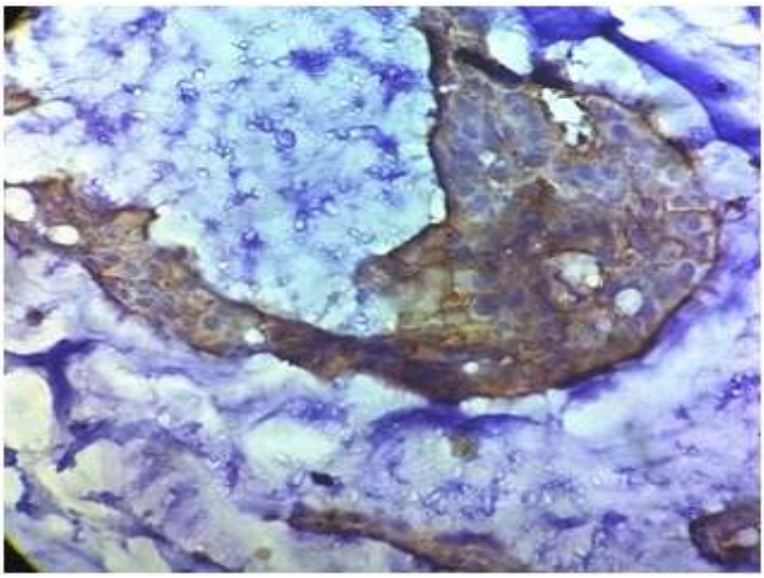
COLOUR PLATE 1:

IMMUNOHISTOCHEMISTRY OF BCL2 IN MUCINOUS CARCINOMA OF BREAST



10X VIEW

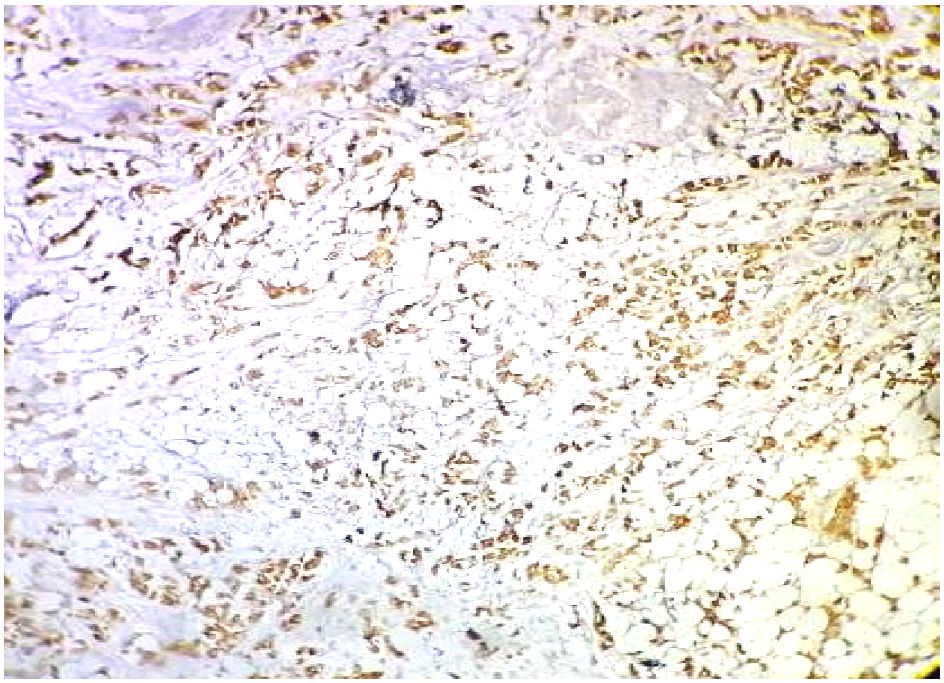
(low power view showing clusters of cells with cytoplasmic expression of bcl2 seen in a background of mucin)



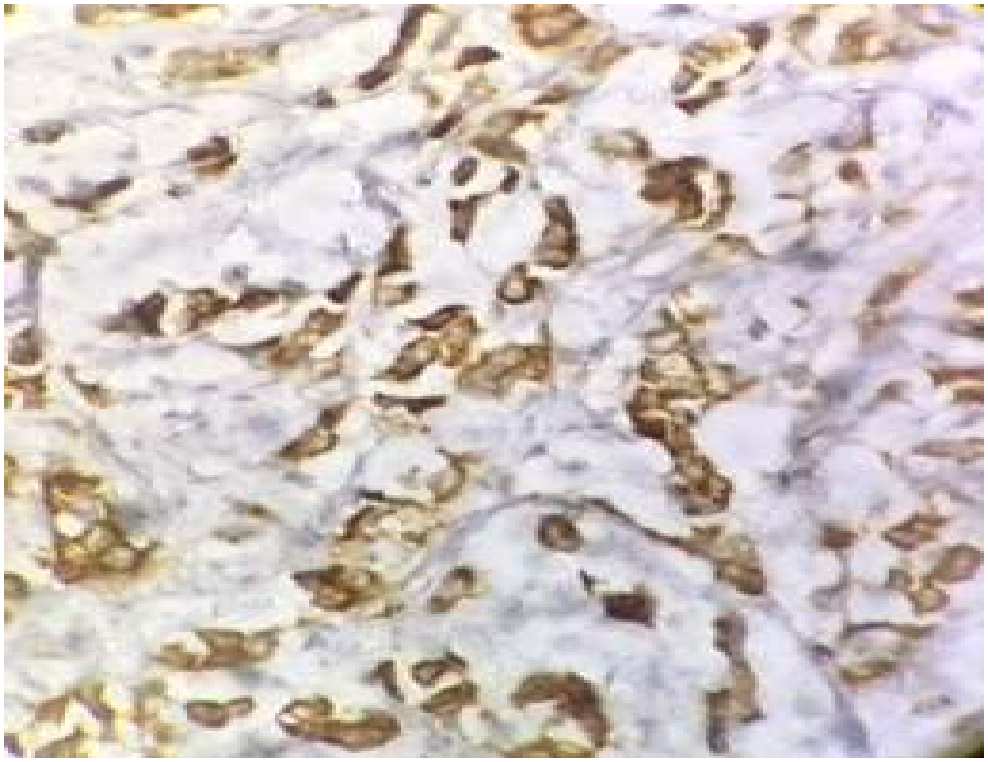
40X VIEW

[high power view showing bcl2 expression.]

2.BCL2 EXPRESSION IN DUCTAL CARCINOMA OF BREAST

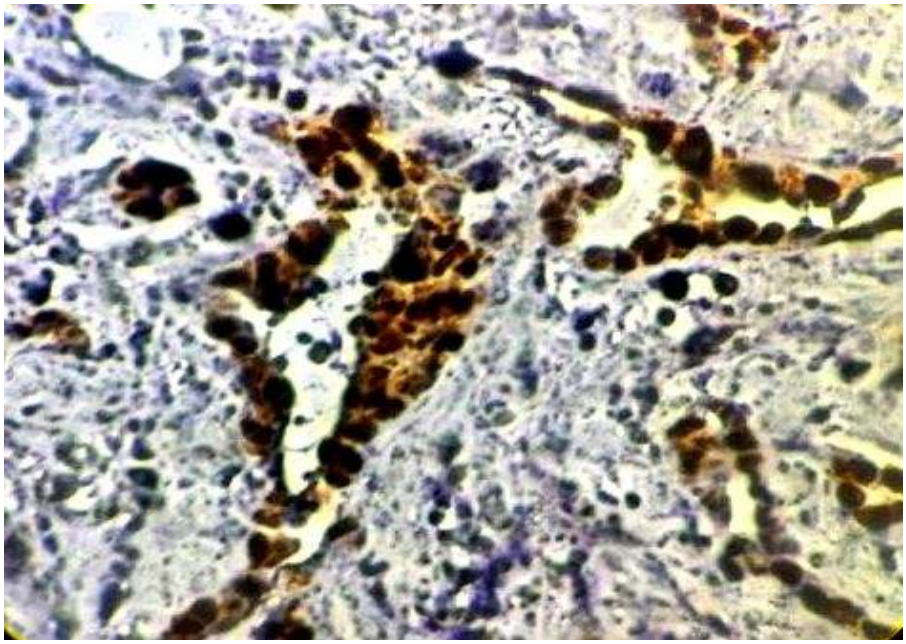


LOW POWER VIEW 10X



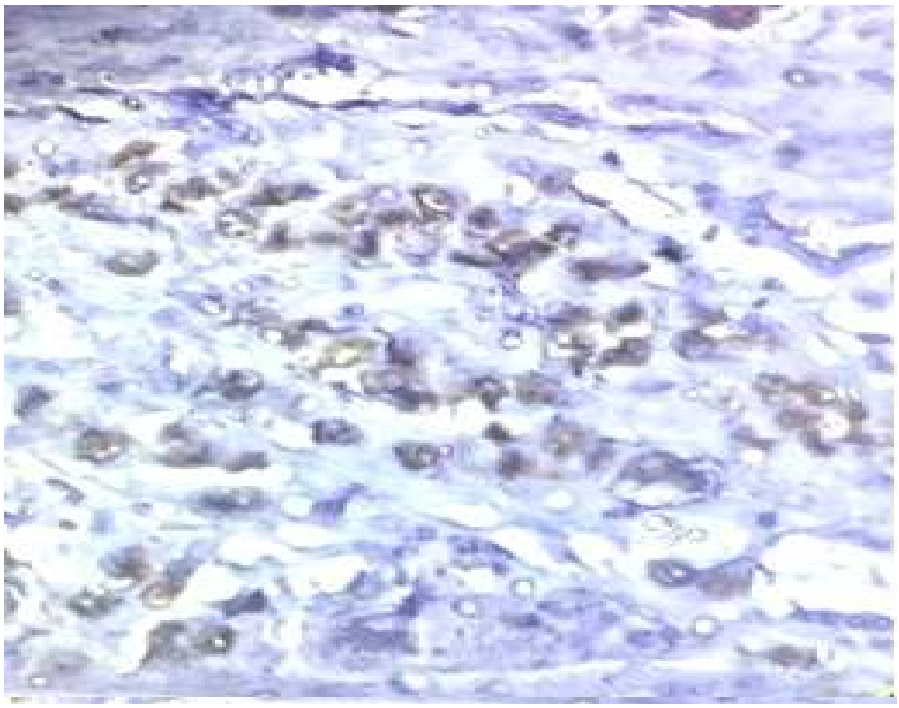
HIGH POWER VIEW 40X

3.IMMUNOHISTOCHEMISTRY OF ER POSITIVITY



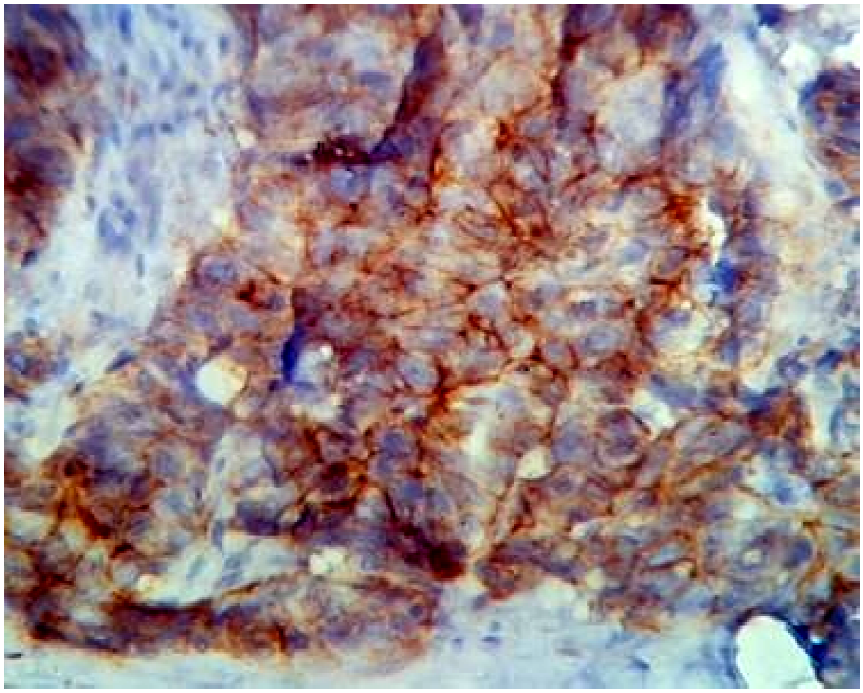
Picture showing ER nuclear positivity(8/8)

4.IMMUNOHISTOCHEMISTRY OF PR POSITIVITY



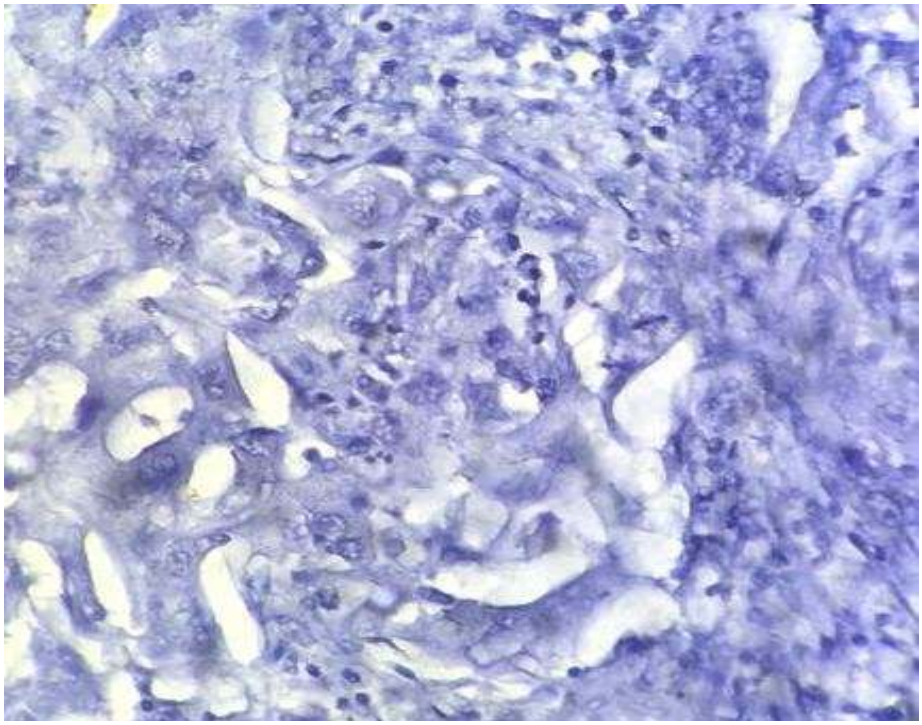
Picture showing PR nuclear positivity(5/8)

5.IMMUNOHISTOCHEMISTRY OF HER2/NEU POSITIVITY

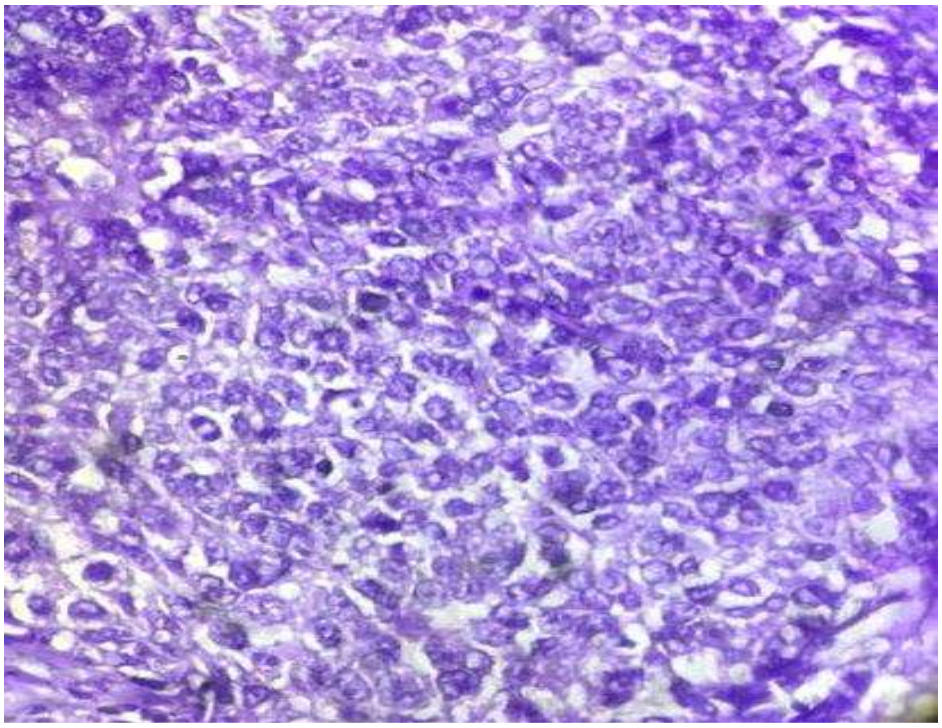


Picture showing HER2/neu Weak to moderate complete membrane staining positivity(2+)

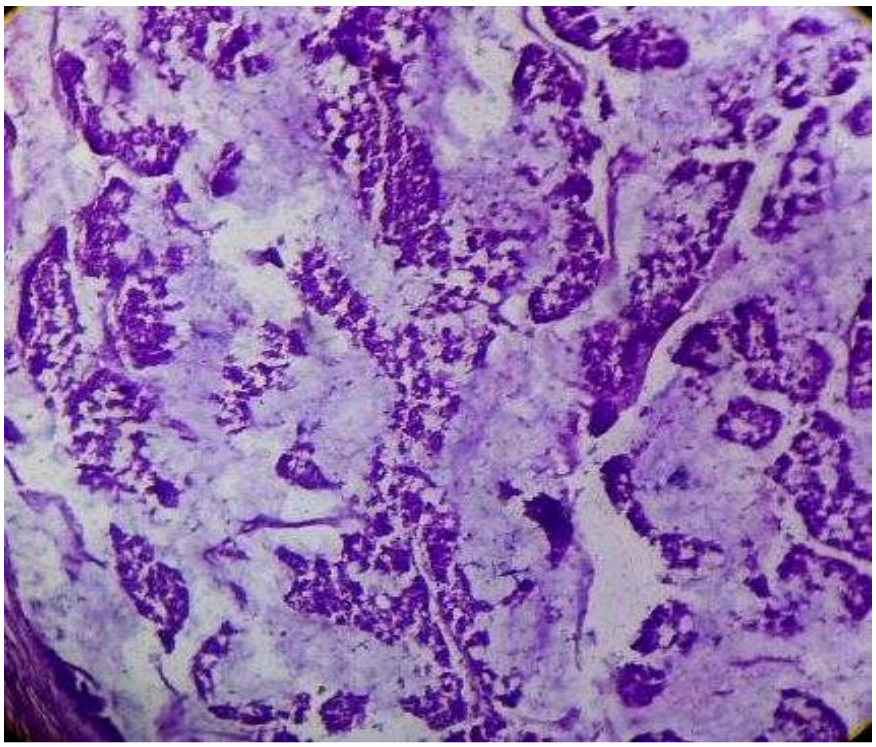
6.IMMUNOHISTICHEMISTRY PICTURE OF BCL2 NEGATIVITY



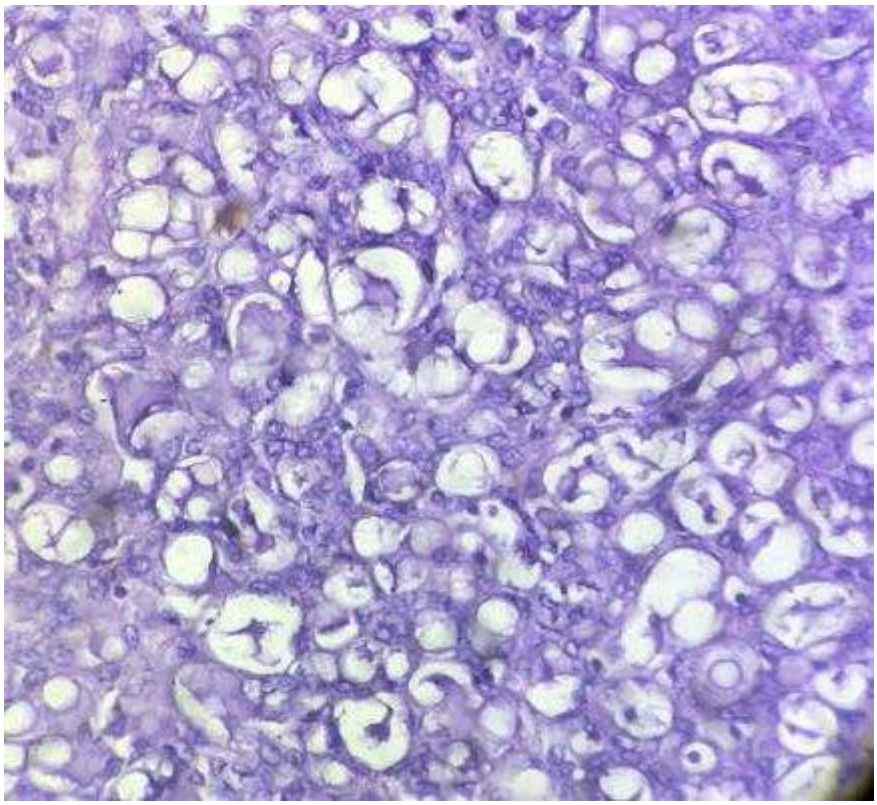
7.H&E PICTURE OF DUCTAL CARCINOMA OF BREAST



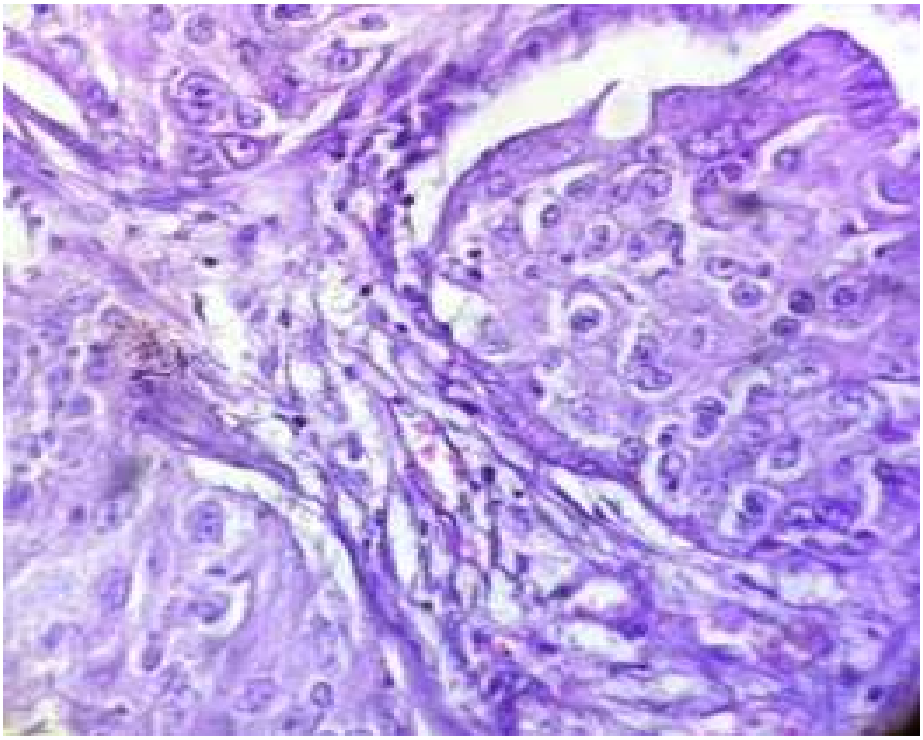
8.H&E PICTURE OF MUCINOUS CARCINOMA OF BREAST



9.H&E PICTURE OF SECRETORY CARCINOMA(40X)



10.H&E PICTURE OF METAPLASTIC CARCINOMA(HIGHPOWER)40X



DISCUSSION:

INCIDENCE:

Invasive ductal carcinoma of breast is the third most frequent carcinoma reported in the Department of Pathology, Coimbatore Medical College and accounts for 10.3% of total malignancies in the year 2017.

AGE OF OCCURENCE:

In our study majority of invasive ductal carcinoma of breast cases were between 35 and 76 years. The mean age of invasive ductal carcinoma of breast in this study was 52 years. Hwang et al found 48.75 years as mean age of patients in their study.Abdel fatah et al in his study found that median age was 51 years.[44]

DISTRIBUTION OF HISTOLOGICAL VARIANTS IN OUR STUDY

IDC-NST type constitutes 83.3% of cases among the various histological variants included in the study.Other special histological types (mucinous,metaplastic and secretory carcinoma) constitute the remaining part of study.

BCL2 EXPRESSION IN OUR STUDY AND COMPARITIVE ANALYSIS:

In this study 23.3% (7 out of 30) of the cases showed positivity for bcl2 expression and all cases were strongly positive .

In a study done by Oakes et al 83%(45out of 54)of luminal tumours of breast showed bcl2 expression [18].Hwang et al also found bcl2 expression in 68.2%(4932/7230) of invasive ductal carcinoma of breast. We found that percentage of bcl2 positive cases decreases with increasing grade.About 45.5% cases of grade1 Ductal ca NST type and 14.3% of cases of grade 2 ductal carcinoma were positive for bcl2 expression.

All mucinous carcinomas showed bcl2 positivity with p value of $p<0.025$(statistically significant) and score 3+ was found in all the cases.Grade3(Poorly differentiated carcinomas) showed no immunoreactivity with bcl2.Joehnsu et al(1994) in his study found that Bcl-2 expression was particularly common in well differentiated carcinomas (83% moderately or strongly positive).

BCL2 EXPRESSION WITH OTHER CLINICOPATHOLOGIC VARIABLES:

Direct correlation between bcl2 expression and tumour size was found in ductal carcinoma NST with significant p value<0.024.This signifies that smaller tumours show more positivity for Bcl2 expression than larger

tumours. For practical purpose according to AJCC TNM STAGING, tumours are segregated into T1-<2cm,T2-2 to 5cm,T3->5cm. Average tumour diameter was more than 2-5cm in patients with absence of bcl2 expression.

Similarly according to AJCC TNM Staging, lymph node status were divided into N0-Absence of metastasis,N1-1 to 3 involved nodes,N2-4-9 nodes positive,N3-10 or more nodes positive. Lymph node status doesn't appear to show significant association with bcl2 expression.

HORMONE RECEPTOR STATUS WITH BCL2 EXPRESSION:

Bcl2 expression was strong and diffuse in all the cases which were strongly positive for ER and PR receptors,with a statiscally significant p value.(<0.04)

Seven cases with bcl2+/ER+/PR+ve phenotype lacked Her2/neu expression.Assessing the BCL2 expression according to molecular classification, we observed that luminal A type and normal breast like tumours expressed bcl2 marker at a rate of 44.4 %,luminal B and remaining molecular subtypes did not show bcl2 expression.Thus the above results reveal that in breast cancer,bcl2 expression is associated with tumours with better differentiation(grade 1 lesions which are ER positive). Most previous studies of Bcl2 in breast cancer have also shown a favourable outcome in bcl2 positive tumours,This association is underscored by the fact that bcl2 is expressed in

normal glandular epithelium and is upregulated by estrogen possibly as a result of direct transcriptional induction with negative regulation by p53-dependant mechanisms.

In our study, we reported strong correlation between the presence of Bcl2 and estrogen receptor positivity,lower histological grade and tumour size in ductal carcinoma NST; no relationship with lymphnode status and inverse relationship with HER2 positivity.

BCL2 EXPRESSION WITH NOTTINGHAM PROGNOSTIC INDEX:

The original Nottingham Prognostic Index is a numerical value which is calculated as follows.

NPI	VALUE	PROGNOSIS	15 YEAR SURVIVAL RATE
1	<3.4	GOOD	80%
2	3.4-5.4	MODERATE	42%
3	>5.4	POOR	13%

NPI=Tumour size in cm*0.2+histological grade(1-3)+number of positive lymphnodes(1=0nodes,2=1-3 nodes,3=>3 nodes)

Kurshumliu et al(2014)observed in their study, a strong correlation between ER and bcl-2 expression, and hence an inverse statistical correlation between bcl-2 and NPI value. This is also supported in the study by Zhang et al. [24], who concluded the following: 1) expression of bcl-2 is associated with

better response to hormonal therapy, and 2) expression of bcl-2 is a good prognostic marker irrespective of the nodal status. Some early studies such as one did by Zhang et al have reported an inverse correlation between expression of bcl-2 and immunohistochemical detection of EGFR, Her-2/neu, and p53 .

COMPARISON BETWEEN OUR STUDY AND PREVIOUS STUDIES

S.no	Name of the study	Sample size	Direct Correlation	Inverse Correlation	No Correlation
1.	Our Study	30	ER,PR Low tumour grade	Her2 exp	Lymph node status
2.	Honma et al 2015	1111	Small tumour size,low grade, ER+,PR+	Her2 exp	Nodal status
3.	Hwang et al 2012	7230	Tumour size<2cm, ER/PR+, grade 1/2	Her2 exp,nodal status, Lymphovascular invasion	-
4.	Slooten et al 1996	441	Low tumour grade, ER/PR+	P53 C-erb b2+ High MIB1 index High mitotic index,large tumour size	Response to chemotherapy
5.	Fatah et al 2013	600	High exp of p27,MDM4 and sperm associated antigen 5.	Triple negative tumours,high mitotic index,high levels p Cadherin,e-Cadherin,CK19 and Her3	

SUMMARY AND CONCLUSION

SUMMARY

A study conducted in Coimbatore Medical College, Coimbatore during the year 2016-2017. The study titled as “EXPRESSION OF BCL2 IN BREAST CARCINOMAS AND ITS CORRELATION WITH OTHER CLINICOPATHOLOGIC VARIABLES SUCH AS NOTTINGHAM PROGNOSTIC INDEX, ER, PR AND HER2 NEU EXPRESSION ”.

The study consists of 30 cases of invasive carcinoma of breast. In all the cases immunohistochemistry was done with markers ER, PR,HER2/neu and Bcl2. Male patients, patients with other malignancies, bilateral breast carcinoma patients are excluded from the study. Statistical analysis was done and results were compared with previously available related studies on Bcl2 expression This study shows:

1. According to malignancy statistics of our department, breast carcinoma constitutes 10.3% of total malignancies during the study period 2016-2017.Out of that 30 cases were randomly selected and study was done.In our study majority of breast carcinoma patients belongs to 35 to 72 age group.
2. Mean age of invasive ductal carcinoma of breast in this study is 52 years.

3. IDC-No specific type constitutes 83.3% of cases among the various histological variants included in the study.Other special histological types (mucinous,metaplastic and secretory carcinoma) constitute the remaining part of study.
4. 7 out of 30 cases was positive for Bcl2 expression,out of which all are strongly positive for Bcl2.
5. ER,PR positivity and Her2/neu negativity is observed in Mucinous carcinoma of breast.
6. Triple negativity is observed in Metaplastic carcinoma and Secretory carcinoma of breast.
7. Her2/neu overexpression is found in 13.3% of cases.Bcl2 shows no immunoreactivity in those cases.
8. Among 30 cases,36.7% belongs to well differentiated histological grade,46.7% moderately differentiated grade,16.7% poorly differentiated grade.
9. Bcl2 expression was found in 45.5 % grade I well differentiated ductal carcinoma ,14.3% of Grade II Moderately differentiated ductal carcinomas,all cases of mucinous carcinoma of breast(p-<0.025)
10. Bcl2 expression was negative in gradeIII or Poorly differentiated Ductal carcinomas,Metaplastic carcinoma,Secretory carcinoma.

11. Bcl2 and ER/PR status found to have a statistically significant direct correlation with p value<0.004.
12. Direct correlation between Bcl2 expression and tumour size is found in ductal carcinoma NST with significant p value<0.024 that signifies smaller tumour shows more positivity for Bcl2 expression than larger size tumours.No statistically significant correlation between lymphnode status and Bcl2 expression can be found out.
13. NPI 1 and Bcl2 expression is found to have direct correlation.

CONCLUSION

To conclude,bcl2 expression in invasive ductal carcinoma is directly correlated with lower histologic grade(well differentiated tumours),small tumour size,ER positivity,PR positivity,all cases of mucinous carcinoma of breast.It inversely correlated with Her2/neu positivity and other special histologic subtypes(metaplastic and secretory carcinoma).

These data promise on a new approach to enhance the efficacy of endocrine treatment in estrogen receptor-positive (ER+) breast cancer by negating the antiapoptotic effect of BCL-2 using BH3 mimetics . Teixeira et al. (1995) reported that ER positive(MCF7 human) breast cancer cells had increased sensitivity to the cytotoxic agent doxorubicin when treated with antisense BCL-2(BH3 mimetics). Lastly, ER+ tumors were found to have a reduced likelihood of complete pathological eradication with chemotherapy or anti HER2 therapies than ER negative tumors. Hence Bcl2 antagonism in these ER+ tumors might increase the effectiveness of these predominately proapoptotic drugs when compared to the conventional treatment.

More recently, a number of BH3 mimetic small molecules, which mimic the action of proapoptotic BH3- only proteins, have been developed to counteract antiapoptotic proteins such as BCL-2 and BCL-2-related proteins like BCL-XL and BCL-W.Further development of bcl2 inhibitors has been explored and small molecule inhibitors such as ABT-737 AND ABT-199 have been recently introduced.Emerging evidence also suggests the usefulness of this

type of therapy in breast cancer.However further studies are needed which involve larger scale of patients to assess Bcl2 expression across different molecular subtypes of breast carcinoma to prove the effectivenesss of new therapy targeting bcl2.

BIBLIOGRAPHY

1. NCRP, Three year report of population based cancer registries 2012-14,ICMR.
2. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1085.
3. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1720.
4. Tawfik K et al.Prognostic significance of bcl2 in invasive mammary carcinomas.Hum Pathol.2012;43:23-30.
5. Ugur Akar et al.Autophagy 4:5,669-679;1 july 2008-Silencing of Bcl2 expression by small interfering RNA induces autophagic cell death in MCF-7 breast cancer cells
6. Wang CW,Klionsky DJ.The molecular mechanism of autophagy.Mol Med 2003;8:65-76
7. KerrJF et al.Apoptosis.Its significance in cancer and cancer theraphy.Cancer 1994;73:2013-26.
8. Pattigre S,Tassa A et al.Bcl2 antiapoptotic proteins inhibit Beclin 1-dependant autophagy.Cell 2005;122:927-39
9. Del Bufalo D et al.Bcl2 overexpression enhances the metastatic potential of a human breast cancer line.FASEB J.1997;11;947-953
10. Ioachim EE et al.Immunohistochemical expression of bcl2 proteins in breast lesios:correlation with Bax,p53,Rb,C-erb2,EGFR and Proliferation indices
11. ..Lee KH et al.Prognostic significance of bcl2 expression in stage iii breast cancer patients who had received doxorubicin and cyclophosphamide followed by paclitaxel as ajuvant chemotheraphy.BMC Cancer.2007;7:63
12. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1086

13. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1094.
14. Rosai and Ackerman's Surgical Pathology, Tenth edition, volume 2, page no: 1720.
15. T.M.A.Abdel fatah et al.Annals of oncology,Vol24,Issue11.1 Nov2013-Bcl2 is an independent prognostic marker of triple negative breast cancer(TNBC) and predicts response to anthracycline combination(ATC) chemotheraphy(CT) in adjuvant and neoadjuvant settings.
16. Ali coombes et al.A Ki67/Bcl2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer.J Pathol 2012;226;97-107
17. 17.Teixira et al.Cancer Res,1995 Sep1;55(17):3902-7-Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl2 proto-oncogene expression in human breast cancer cells
18. Fitzgibbons PL et al.Prognostic factors in breast cancer.Arch Pathol Lab Med 2000;124:966-78
19. Perou CM et al.Molecular portraits of human breast tumours.Nature 2000;406:747-52
20. Fisnik Kurshumliu et al World journal of surgical oncology 2014,12;243- Classification of patients with breast cancer according to Nottingham Prognostic Index highlights significant differences inimmunohistochemical marker expression
21. Lesley-Ann Martin, and Mitch Dowsett et al. BCL-2: A New Therapeutic Target in Estrogen Receptor-Positive Breast Cancer?
22. Blackwood MA, Weber BL. BRCA1 and BRCA2: from molecular genetics to clinical medicine. J Clin Onco 1998,16: 1969-77.
23. Futreal PA et al, BRCA1 mutation in primary breast and ovarian carcinomas. Science 1994,266: 120-122

24. Tan DS, Marchio C, Reis-Filho JS. Hereditary breast cancer: from molecular pathology to tailored therapies. J Clin Pathol 2008,61:1073-1082.
25. Ellis I O, Galea M, Broughton N et al. 1992 Pathological prognostic factors in breast cancer. II. Histological type. Relationships with survival in a large study with long-term follow-up. Histopathology 20: 479-489.
26. Tavassoli F, Devilee P 2003 Pathology and genetics of tumours of the breast and female genital organs. World Health Organization classification of tumours. IARC Press, Lyon, France.
27. Rosen P P, Cranor M L 1991 Secretory carcinoma of the breast. Arch Pathol Lab Med 115: 141-144.
28. Komaki K, Sakamoto G, Sugano H et al. 1988 Mucinous carcinoma of the breast in Japan. A prognostic analysis based on morphologic features. Cancer 61: 989-996.
29.. Rosai and Ackerman's Surgical pathology ,Tenth edition, Volume 2, page number 1689
30. Rosai and Ackerman's Surgical pathology ,Tenth edition, Volume 2, page number 1690
31. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition, page no: 1079.
32. Susan C. Lester, Robbins and Cotran Pathologic basis of Disease, Eighth edition page no:1080
33. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1088.
34. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1096.
35. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1097

36. Henry J.Norris,Herbert B.Taylor.Cancer.Vol18,Issue7,July 1965,pages 879-885.Prognosis of Mucinous(Gelatinous) carcinoma of the breast
37. Rosen P P, Cranor M L 1991 Secretory carcinoma of the breast. Arch Pathol Lab Med 115: 141-144.
38. Zhang GJ,Tsuda H et al.Prognostic indicators for breast cancer patients with one to three regional lymph node metastases,with special reference to alterations in expression levels of bcl2,p53,c-erbB2 proteins.Jpn J Clin Oncol 1997;27:371-7
39. Tawfik K et al.Prognostic significance of Bcl2 in invasive mammary carcinomas:a comparative clinicopathologic study between triple negative and non triple negative tumors.Hum Pathol.2012;43:23-30.
40. P Hellemans et al.British journal of cancer(1995)72,354-360-Prognostic value of Bcl2 expression in invasive breast cancer
41. Grace M.callagy,Paul D.Pharoah et al.Clin Cancer Res 2006;12(8)april15,2006-Bcl2 is a prognostic marker in breast cancer indepndantly of the Nottingham prognostic index
42. .Application of immunohistochemistry in breast pathology-Haiyan liu MD,Arch Pathol Lab Med. 2014;138:1629-1642
43. B M SYED, A R GREEN ,E C PAISH et al,British journal of cancer(2013)108,1042- 1051 on Biology of primary breast cancer in older women treated by surgery:with correlation with longterm clinical outcome and comparison with their younger counterparts
44. Honma et al.BMC Cancer(2015)15:698-Differences in clinical importance of bcl2 in breast cancer according to harmone receptor status or adjuvant endocrine theraphy
45. Banu arun,Gokhan kilic et al .Cancer 2003;98:2554-9-Correlation of bcl2 and p53 Expression in primary breast tumours and corresponding Metastatic lymph nodes

46. Dana Carmen et al.Rom J Morphol Embryol 2012,53(1):155-160-Molecular characterization of apoptosis by the immunohistochemical evaluation of BCL2 in breast cancer
47. Mee seon Kim et al.Int J Clin Pathol 2015;8(8):8842-8856.Expression of alpha B crystallin and Bcl2 in patients with infiltrating ductal carcinoma
48. Kapil dev et al.International Journal of Advanced Scientific andTechnical Research Issue3 volume6,Nov-Dec2013-Expression of anti apoptotic protein Bcl2 and its correlation with histological grade,age in breast cancer
49. H J van slooten et al.British journal of cancer(1996)74,78-85-Expression of BCL2 in node negative breast cancer is associated with various prognostic factors,but does not predict reponse to onecourse of perioperative chemotheraphy
50. M G Daidone,A Luisi et al.Endocrine –Related Cancer(1999)61-68-Clinical studies of BCL2 and treatment benefit in breast cancer patients.
51. Giampietro Gasparini et al.Clinical cancer research Vol 1,189-198,February 1995.-Expression of bcl2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer.
52. Lerwill MF.Current practical applications of diagnostic immunohistochemistry in breast pathology.Am J Surg Pathol.2004;28(8):1076-1091.
53. Christopher. D. M Fletcher Diagnostic Histopathology of Tumor, Fourth edition, Vol 1, page no:1094.
54. Lipponen P et al.Apoptosis suppressing protein bcl2 is expressed in well differentiated breast carcinomas with fauvourable prognosis.J Pathol 1995;177:49-55
55. Barbareschi M et al.Bcl2 and p53 expression in node negative breast carcinoma: a study with long term follow up.Hum Pathol 1996;27:1149-5

56. Simona Daniela Neamtu, Iulia Tudorascu et al. BCL2 gene expression in breast tumours. *Current Health Sciences Journal Vol. 39, No. 2, 2013 April June*

57. Manabu Emi, Ryungsa Kim et alTargeted therapy against Bcl-2-related proteins in breast cancer Cells.Breast cancer research 2005, 7:R940-R952

58. Huang Z: Bcl-2 family proteins as targets for anticancer drug design. Oncogene 2000, 19:6627-6631.

59. Leek RD, Kaklamanis L, Pezzella F, Gatter KC, HarrisAL: Bcl-2 in normal breast and carcinoma, association with oestrogen receptor-positive, epidermal growth factor receptor-negative tumours and in situ cancer.Br J Cancer 1994, 69:135-139

60. Nathan B et al.Expression of bcl2 like immunoreactivity in the normal breast and breast cancer.Breast 2:134-137,1993.

61. Nathan B et al,Expression of bcl2 in Primary breast cancer and its correlation with tumour phenotype.Ann.Oncol.,5:409-414,1994

62. Joensuu H et al(1994).Bcl2 protein expression and long term survival in breast cancer.Am J Pathol.,145,1191-1198.

63. Sabourin JC et al(1994).Bcl2 expression in normal breast tissue during the menstrual cycle.Int.J.Cancer,59,1-6.

64. Kirkin V et al.The role of Bcl2 family members in tumorigenesis.Biochim Biophys Acta.2004;1644:229-49

65. Yang D,Chen MB et al.Bcl2 expression predicts sensitivity to chemotheraphy in breast cancer:a systematic review and meta analysis.J Exp Clin Cancer Res.2013;32:105

66. Souers AJ et al.ABT-199,a potent and selective Bcl2 inhibitor,achieves antitumour activity while sparing platelets.Nat Med.2013;19:202-8

67. Bhargava V,Kell DL et al.Bcl2 immunoreactivity in breast carcinoma correlates with harmone receptor positivity.Am J Pathol 1994;45:535-40

68. Ki-Tae Hwang et al.Prognostic influence of BCL2 expression in breast cancer.Int J.Cancer :131E1109-1119(2012)
69. Samantha R.Oakes et al 2012 Sensitization of Bcl2 expressing breast tumors to chemotheraphy by the BH3 mimetic ABT-737
70. Tse C et al.(2008) ABT-263:A potent and orally bioavailable Bcl2 family inhibitor.Cancer Res 68:3421-3428
71. Adams JM,Cory et al(2007).The Bcl2 apoptotic switch in cancer development and theraphy.Oncogene 26:1324-1337
72. .Knowlton K et al(1998).Bcl2 slows in vitro breast cancer growth despite its antiapoptotic effect,J Surg Res,1998,76(1):22-26.
73. S.J.Dawson et al.British journal of cancer (2010)103,668-675-BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received

ANNEXURE 1:PROFORMA

Name age
Ward IP NO:
Address

Presenting complaints

Lump in breast
Pain

Discharge from nipple

Skin ulceration
Duration of presenting illness

Past history

History of previous surgeries for breast lump
History of chemotherapy/radiotherapy

History of breast lump in other breast
Family history
Personal history

Diet
Menstrual history
Breast feeding history

General examination

Nourishment

Built
Conscious
Febrile/afebrile
Pallor
Jaundice
Cyanosis
Clubbing
Lymphadenopathy

Edema
Vitals
PR
RR

BP
Local examination of the breast

Side – right/left
Quadrant
Size of the tumor
Fixity to the skin

Fixity to the underlying fascia
Examination of axillary lymph node
Number of node
Mobile/fixed
Size
Group of node: anterior/posterior/lateral/apical

Gross examination of modified radical mastectomy specimen:

Size of the specimen including skin, nipple, areola
Size of the tumor
Margins: infiltrative/circumscribed
Quadrant

Histological diagnosis

Any special type:
Lymph node status – no: of positive nodes/no: of total nodes examined
Histological grading
Tubule formation: 1/2/3
Nuclear pleomorphism:1/2/3
Mitosis:1/2/3Histological grade: I/II/III

ANNEXURE 2:GLOSSARY

ABBREVIATIONS

AJCC	American joint committee classification
WHO	World HealthOrganisation
CEA	Carcinoembryonicantigen
CI	Confidence Interval
DAB	Diaminobenzidine
DCIS	Ductal carcinoma in situ
DPX	Dextrene polystyrene xylene
ER	Estrogen receptor
GCDFP	Gross Cystic Disease Fluid Protein
HER2	Human epidermal growth factor receptor
NPI	Nottingham prognostic index
LCIS	Lobular carcinoma in situ
NOS	Not Otherwise Specified
PR	Progesterone receptor
TBS	Tris buffer solution
P value	Probability value

ANNEXURE 3: MASTER CHART

NAME	hpe no	ip num	age	hpe diagnosis	stage	er status	pr status	HER2 status	tumour size	LN STATUS	GRADE	LATERALITY	BCL2 EXP	NPI SCORE	NPI VALUE	NPI PROGNOSIS
ROOTH	472/17	5318	60/F	IDC-NOS	PT1NOMX	POSITIVE	POSITIVE	NEGATIVE	T1	NO	GRADE1	RIGHT	POSITIVE	1	2.1	GOOD
KANJANA	4210/16	515863	40/F	IDC-NOS	PT2N2MX	POSITIVE	NEGATIVE	NEGATIVE	T2	NO	GRADE2	RIGHT	NEGATIVE	1	2.6	GOOD
VIJAYA	305/17	2012	56/F	IDC-NOS	PT1N3MX	NEGATIVE	POSITIVE	NEGATIVE	T1	N3	GRADE1	RIGHT	NEGATIVE	3	4.2	MODERATE
DEIVANAI	464/17	6391	54/F	IDC-NOS	PT3N1MX	NEGATIVE	POSITIVE	NEGATIVE	T3	N1	GRADE2	RIGHT	NEGATIVE	3	5.9	POOR
BALKISH	1748/16	781	39/F	IDC-NOS	PT3N3MX	NEGATIVE	NEGATIVE	POSITIVE	T3	N3	GRADE3	RIGHT	NEGATIVE	3	7.2	POOR
MAHESWARI	647/17	8800	41/F	MUCINOUS CA	PT2NOMX	POSITIVE	POSITIVE	NEGATIVE	T3	NO	GRADE1	LEFT	POSITIVE	1	1.4	GOOD
MEBSIBA	388/17	4914	38/F	IDC-NOS	PT2N3MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N3	GRADE1	LEFT	NEGATIVE	3	5.96	POOR
SHANTHA	872/17	11101	67/F	IDC-NOS	PT2N2MX	NEGATIVE	NEGATIVE	NEGATIVE	T3	N2	GRADE1	RIGHT	NEGATIVE	2	4	MODERATE
PALANIYAMMAL	1826/16	36645	71/F	IDC-NOS	PT2N0MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N0	GRADE2	RIGHT	NEGATIVE	1	2.8	GOOD
CHINNAMMAL	2789/16	56533	56/F	IDC-NOS	PT3N2MX	NEGATIVE	NEGATIVE	POSITIVE	T3	N2	GRADE2	RIGHT	NEGATIVE	3	6	POOR
SAKILABANU	572/17	6367	40/F	IDC-NOS	PT3N2MX	NEGATIVE	NEGATIVE	NEGATIVE	T3	N2	GRADE1	LEFT	NEGATIVE	3	5.8	POOR
THULASI	807/17	15372	55/F	IDC-NOS	PT2N0MX	POSITIVE	NEGATIVE	NEGATIVE	T2	NO	GRADE2	RIGHT	NEGATIVE	1	2.6	GOOD
KANDIYAMMAL	1218/17	21593	67/F	MUCINOUS CA	PT3N1MX	POSITIVE	POSITIVE	NEGATIVE	T3	N1	GRADE1	LEFT	POSITIVE	1	3.2	GOOD
SELVAJOTHI	2766/17	81715	39/F	IDC-NOS	PT3N1MX	NEGATIVE	NEGATIVE	NEGATIVE	T3	N1	GRADE2	RIGHT	NEGATIVE	2	4.4	MODERATE
UMA	2717/17	84009	40/F	IDC-NOS	PT2N2MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N2	GRADE3	LEFT	NEGATIVE	3	6.68	POOR
KAMMALI	2783/17	81458	52/F	SECRETORY CARCINOMA	PT1NOMX	NEGATIVE	NEGATIVE	NEGATIVE	T1	NO	GRADE3	LEFT	NEGATIVE	2	4.3	MODERATE

SUMA	2811/17	86512	54/F	MUCINOUS CA	PT2N0MX	POSITIVE	POSITIVE	NEGATIVE	T2	N0	GRADE1	RIGHT	POSITIVE	1	2	GOOD
JOTHIMANI	2857/17	86540	52/F	IDC-NOS	PT3N3MX	NEGATIVE	NEGATIVE	POSITIVE	T3	N3	GRADE2	LEFT	NEGATIVE	3	7.4	POOR
RANI	2917/17	90688	50/F	IDC-NOS	PT3N3MX	NEGATIVE	NEGATIVE	NEGATIVE	T3	N3	GRADE3	RIGHT	NEGATIVE	3	7.6	POOR
KRISHNAVENI	2912/17	50119	40/F	IDC-NOS	PT1N2MX	POSITIVE	POSITIVE	NEGATIVE	T1	N2	GRADE2	LEFT	POSITIVE	2	5.24	MODERATE
AISWARYAMMAL	4048/16	76531	76/F	IDC-NOS	PT2N2MX	POSITIVE	POSITIVE	NEGATIVE	T2	N2	GRADE2	LEFT	positive	3	5.4	MODERATE
TAMILARASI	4114/16	76169	44/F	IDC-NOS	PT2N2MX	POSITIVE	POSITIVE	NEGATIVE	T2	N2	GRADE2	RIGHT	NEGATIVE	3	5.4	MODERATE
PANKAJAM	1353/15	26067	72/F	IDC-NOS	PT2N2MX	POSITIVE	POSITIVE	NEGATIVE	T2	N2	GRADE1	RIGHT	NEGATIVE	3	4.8	MODERATE
KALIYAMMAL	2031/17	39104	57/F	IDC-NOS	PT2N1MX	NEGATIVE	NEGATIVE	POSITIVE	T2	N1	GRADE2	LEFT	NEGATIVE	3	4.6	MODERATE
VALARMATHY	2431/17	47540	50/F	IDC-NOS	PT3N0MX	NEGATIVE	NEGATIVE	NEGATIVE	T3	NO	GRADE2	RIGHT	NEGATIVE	1	3.2	GOOD
SEETHALAKSHMI	823/17	13323	59/F	IDC-NOS	PT2N1MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N1	GRADE1	RIGHT	NEGATIVE	1	2.9	GOOD
SIVAGAMI	1285/17	22496	65/F	METAPLASTIC CARCINOMA	PT2N0MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N0	GRADE3	RIGHT	NEGATIVE	2	3.7	MODERATE
LAKSHMI	4116/16	77882	70/F	IDC-NOS	PT2N1MX	NEGATIVE	NEGATIVE	NEGATIVE	T2	N1	GRADE2	LEFT	NEGATIVE	2	4	MODERATE
RAJAMANI	4247/16	80369	42/F	IDC-NOS	PT2NIMX	POSITIVE	POSITIVE	NEGATIVE	T2	N1	GRADE2	RIGHT	NEGATIVE	3	6	POOR
PAPPAL	309/17	4905	35F	IDC-NOS	PT2N1MX	POSITIVE	POSITIVE	NEGATIVE	T2	N1	GRADE1	RIGHT	POSITIVE	1	2.8	GOOD